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Impact of Purification on Physicochemical, Surface and Functional Properties of Okra Biopolymer

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Abstract:

The functionality of plant-resourced biopolymers depends on their physicochemical properties. In addition, the extraction protocol and further processing conditions can significantly affect the effectiveness of biopolymer in diverse industrial applications. Therefore, the objective of this study was to investigate the impact of purification on chemical composition, molecular arrangement, solubility, swelling, erosion, wettability, quantitative wetting kinetics, surface energy and three-dimensional (3D) surface texture properties of okra biopolymer and its compacted form. FTIR and XRD results confirmed that the purification process had no effect on the molecular structural arrangement. The highest purity grade (bi-purified okra biopolymer) had the highest sugar content, solubility, matrix tablet swelling, wettability and surface energy, although the surface porosity and roughness of matrix tablet were low. Okra biopolymer showed pH-dependent solubility and the maximum solubility was achieved at pH 7.4. The mechanism of swelling of less-purified matrices was anomalous, where the rate of water diffusion and polymer relaxation was of the same magnitude, whereas bi-purified matrices showed diffusion-controlled swelling. Wetting was absorption-controlled and the bi-purified biopolymer had a high degree of wetting and surface energy. The extraction method, therefore, has a major influence on the properties and the subsequent drug delivery, biotechnology and food science applications for the biopolymer.

Keywords:

Okra biopolymer; Purification; Matrix tablet: Swelling; Erosion; Surface energy

1. Introduction

Natural polymers are composite biomaterials and are commonly resourced from plant, animal and microbial sources (Reis et al., 2008). There are many examples, including, but not limited to, gum arabic (plant exudates), alginate (seaweed), xanthan gum (bacteria), okra biopolymer (fruit), grewia gum (bark), chitin (exoskeletons of arthropods) and chondroitin sulphate (animal cartilage) (John & Thomas, 2012). The potential of natural polymers, especially plant biopolymers, has significantly increased, as confirmed by the enormous quantity of associated papers and patents recently published (Beneke, Viljoen, & Hamman, 2009; Hamman, Steenekamp, & Hamman, 2015; Thakur & Voicu, 2016). Plant-based biopolymers, or modifications thereof, are widely used in pharmaceutical, food and biomedical applications (Alba, Ritzoulis, Georgiadis, & Kontogiorgos, 2013; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014). Most of them are safe for oral consumption and are preferred over their synthetic counterparts because of low cost, non-toxicity and abundant availability (John & Thomas, 2012).

However, there are some technical restrictions regarding their application as they must have to meet the standards of reproducibility, content and purity (Lai & Chen, 2012). As the biopolymers can exist in structurally complex mixtures, which may vary according to location and season, standard isolation and purification techniques are required to ensure the resource can be produced and used in a sustainable manner. This is of special significance when the biopolymer is used at a high concentration to fulfil its anticipated function, such as a modified release tablet matrix former (Beneke, Viljoen, & Hamman, 2009; Amid & Mirhosseini, 2012). Hence, it seems necessary that these biomaterials must undergo a sophisticated purification protocol for the standardisation of their

physicochemical and functional properties. Attributable to naturally occurring sources, these materials generally contain a lot of undesirable substances which can be fibres, proteins, fatty acids, cellulose, hemicellulose, endotoxins (pyrogen) and mitogenic compounds (Vidal-Serp & Wandrey, 2005). These impurities can instigate some serious health concerns in humans. This was highlighted in a series of studies on alginate beads in which beads were formed using highly purified alginate were more biocompatible than commercially available non-purified alginates (De Vos, De Haan, Wolters, Strubbe, & Van Schilfgaarde, 1997; Dusseault et al., 2006; Sharma & Gupta, 2002). Furthermore, another study also revealed that the use of substantially purified alginate significantly reduced fibrotic overgrowth around implants (Qi, Lu, Zhou, & Luo, 2009). Recently, our group has affirmed the potential of purified grewia gum (starch-free) in controlled release applications. It was found that purified grewia gum had comparable potential to the frequently used semi-synthetic polymer, hypromellose (HPMC) in some pharmaceutical applications and may be a useful substitute in countries where it is naturally abundant or can be cultivated easily (Ghori & Conway, 2015; Ghori, Ginting, Smith, & Conway, 2014; Nep, Asare-Addo, Ghori, Conway, & Smith, 2015). The practical production of biopolymers, in amounts compatible with many industrial applications, and with a high degree of purity, would enable their more widespread adoption.

In this study, okra biopolymer was chosen as a model material since it has documented applications across many pharmaceutical, food and biomedical applications (Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014). The application of okra biopolymer as a drug release modifier, film-former, scaffold for tissue engineering, emulsion stabiliser, anti-static agent, tablet coating agent and compressibility enhancer have all been reported recently (Alba,

Ritzoulis, Georgiadis, & Kontogiorgos, 2013; Dimopoulou, Ritzoulis, Papastergiadis, & Panayiotou, 2014; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014; Ghori, Green, Smith & Conway, 2013; Ogaji & Nnoli, 2010). Okra biopolymer is normally acquired from its pods (*Abelmoschus esculentus L.*) and it is widely cultivated in the tropics, sub-tropical and temperate regions around the world including Africa, Asia and North-America. It has a net global production of 6 million tons ha^{-1} and total trade of more than $5 billion (Kontogiorgos, Margelou, Georgiadis, & Ritzoulis, 2012). Extracts of okra are reported as comprising an acidic polysaccharide consisting of different sugars including galactose, rhamnose, galacturonic acid, galactose, glucose and glucuronic acid (Alba, Ritzoulis, Georgiadis, & Kontogiorgos, 2013).

Polymer hydration, solubility and erosion/dissolution are important areas for investigation because of their impact on applications in industry such as biotechnology, food sciences and drug delivery (Miller-Chou & Koenig, 2003; Narasimhan, 2001). Unlike non-polymeric materials, polymers do not dissolve instantaneously, and the dissolution is controlled by either the disentanglement of the polymer chains or by the diffusion of the chains through a boundary layer adjacent to the polymer–solvent interface (Crompton, 2006; Miller-Chou & Koenig, 2003). Moreover, within various healthcare (e.g., drug delivery and tissue engineering) and food science applications, it is vitally important to understand the polymer solubility/dissolution and hydration behaviour. To identify the suitability of biopolymers for the aforementioned industrial applications, one has to evaluate some key performance indicators which include (however, are not limited to) solubility, swelling, erosion, surface wetting, energetics and texture. This paper describes a systematic approach to clarify the

impact of purification on these properties of a biopolymer and how they may impact performance.

Therefore, this study was designed to assess the impact of purification on chemical constituents, solubility, swelling, erosion, wetting, surface energetics and texture of okra biopolymer. Moreover, okra is used as an example of a plant-derived biopolymer model and it is intuitively expected that changes due to the purification process may be extrapolated to similar plant-derived polysaccharide biopolymers expected to be utilised in drug delivery, biotechnology and food science applications. Specific changes due to methods of purification and experimental conditions may, of course, vary from biopolymer to biopolymer.

2. Materials and methods

2.1. Materials

Fresh okra pods were purchased from a local market and were frozen and kept at -15 °C until handling. Sulphuric acid, hydrochloric acid, ethanol and phenol were purchased from Sigma-Aldrich, UK. Diiodomethane (>99% pure), disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Fisher Scientific UK. All reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of okra biopolymer

The extraction of biopolymer from fresh okra pods was carried out according to the details described in Fig. 1. It resulted in three different polymer grades, classified as crude, purified

and bi-purified okra biopolymer. Once dried, the particle size fractions (150-250 μm) were isolated by sieving and used in all subsequent studies.

2.2.2. Chemical analysis

The total carbohydrate content was determined using a phenol-sulphuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and protein content was quantified using a Bradford assay (Bradford, 1976).

2.2.3- Fourier transform infrared (FTIR) analysis

Fourier transform infrared (FTIR) spectra of all the okra biopolymer powder samples (crude, purified and bi-purified) were generated by scanning from 400 - 4000 cm^{-1} at ambient temperature (20.5 °C) using a Thermo Nicolet 380 FTIR with Diamond ATR.

2.2.4- X-ray diffraction analysis

Powder X-ray diffraction of all the okra biopolymer powder samples (crude, purified and bi-purified) was carried out using a D2-Phase X-ray diffractometer (Bruker UK Ltd., Coventry, UK) equipped with a CuKα radiation source at 30 KV voltage and 10 mA current. Diffraction patterns were obtained in the 2θ range of 5°–100° using 0.02 step sizes.

2.2.5. Solubility studies

Solubility of different grades of okra biopolymer was determined using a shake-flask method. Sodium phosphate buffer (0.2 M) was used as the medium and pH of the buffer was measured and adjusted, if necessary, with either diluted phosphoric acid or sodium hydroxide to attain the desired pH values, i.e. 1.2, 2, 3, 4, 5, 5.5, 6.8, 7.4, 8, 10 and 12. Each okra biopolymer variant was added in excess (1 g) to 100 ml of phosphate buffer in glass

vials at 37 ± 0.5 °C. The glass vials were placed in the temperature-controlled shaking apparatus (GLS 12 aqua®) at an agitation speed of 100 rpm. For every grade, three aliquots were prepared at each pH value. After the requisite shaking time, samples were equilibrated for 24 h at 37 ± 0.5 °C, and then the supernatant was filtered through Millex-LH membrane 0.45 µm pore size filters (Millipore®). The pH of the supernatant solutions was then measured. The concentration of okra biopolymer in the supernatant was determined using a phenol-sulphuric acid assay. The quantification method for the polysaccharide-based polymer in their dissolved state has previously been reported by our group (Ghori, 2014; Ghori, Ginting, Smith, & Conway, 2014). Briefly, filtered samples (1 mL) were added to 1 ml of 5% phenol in 0.1 M hydrochloric acid, followed by 5 ml of concentrated sulphuric acid. The resultant solution was mixed vigorously for 10 minutes and placed in a water bath at 25–30 °C for 20 minutes. Absorbance was measured at a maximum wavelength (λ max) of 472 nm and dissolved okra biopolymer content was quantified using a standard calibration curve constructed for each respective grade, so content was determined relative to the grade of polymer used.

2.2.6. Preparation of matrix tablets

All the okra biopolymer powder samples were compressed using a Testometric M500 – 50 CT (Testometric Company Ltd., United Kingdom) materials testing machine equipped with a 13.00 mm Atlas Evacuable Tablet Die (Specac Limited, United Kingdom). The powder was accurately weighed (500 ± 2.5 mg) on an analytical balance and manually poured into the die. Using flat-faced punches, the lower punch was held stationary while the upper punch moved at a speed of 3 mm/min during loading and 3 mm/min on unloading. The compacts were fabricated at an applied pressure of 100 MPa. After ejection, the tablets were stored

over silica gel for 24 h to allow for elastic recovery before any further investigation. Relative humidity and temperature during compaction work were in the range 22–48 % RH and 20-27 °C, respectively. The out-of-die porosity was calculated using Eq. 1.

$$\varepsilon\,(\%) \;= \left(1 - \frac{W}{\rho\left(\frac{d}{2}\right)^{2}\pi T}\right) \times 100 \quad \text{(Eq 1)}$$

where *W, T, d* are weight, thickness and diameter of the matrix tablets, respectively. True density of okra biopolymer powders (ρ) was determined (n = 10), using the AccuPyc 1340 II Pycnometer (Micromeritic, UK) with helium as the inert gas.

2.2.7. Surface roughness studies

2.2.7.1- Atomic force microscopy

The atomic force microscopy (AFM) images were collected using contact mode and a standard optical lever method with a small offset of force using Dimension Icon by Bruker, UK. The height variation in the resulting topography maps is represented by a colour scheme and the topographical information can be reliably inferred from the given colour scheme. The three-dimensional root mean square roughness (Sq) (Eq. 2) (Blunt & Jiang, 2003; Farris, Introzzi, Biagioni, Holz, Schiraldi, & Piergiovanni, 2011) was also determined using SURFSTAND® software (University of Huddersfield) (Blunt & Jiang, 2003). The scan area was 10 × 10 µm^2 and each measurement was carried out in triplicate (n=3)

$$Sq = \sqrt{\frac{1}{MN}\sum_{j=1}^{N}\sum_{i=1}^{M} n^2\,(x_i, y_i)} \qquad \text{(Eq 2)}$$

2.2.8. Swelling studies

Swelling of okra biopolymer base matrix tablets was determined using USP apparatus I, SR II 6-flask (Hanson Research, USA) at 100 rpm at 37 °C. The swelling media were pH 1.2 and pH 7.4 sodium phosphate buffers and the pre-weighed matrix tablet (W_i) of each okra biopolymer variant was immersed in the respective swelling medium (900 ml). The previously weighed baskets, containing hydrated matrix tablets, were removed, lightly blotted with 125 mm filter paper (Whatman®, UK) to remove excess liquid, re-weighed (W_s) and were rapidly replaced back into the swelling media in dissolution apparatus. The mean weight was determined for each formulation and degree of swelling (S) was calculated by using Eq. 3 (Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014)

$$S = \frac{Ws - Wi}{Wi} \times 100 \qquad \text{(Eq 3)}$$

where W_i and W_s are the initial dry and swollen weight of the matrix tablet, respectively, at immersion time (t) in the swelling media. The degree of swelling was determined from the mean of three replicates and presented as degree of swelling (S, %) against time (t).

2.2.9. Erosion studies

The erosion studies were carried out on all okra biopolymer based matrices. The dissolved okra was quantified by adopting a method described in section 2.2.5 (Ghori, 2014; Ghori, Ginting, Smith, & Conway, 2014). The degree of erosion (E, %) was determined from the mean of three replicates and plotted against time (t).

2.2.10. Contact angle and surface wettability

The contact angles of water and diiodomethane were determined for all the variants of okra biopolymer matrices using the sessile drop method. The OCA15plus (Dataphysics, Germany) apparatus was used to capture the contact angle data and further SCA20 software (Data

Physics, Germany) was used for data analysis. The droplets of liquid (0.5 µl) were released from a micro-syringe from a constant height (1 cm) for consistency purposes. The variations in the contact angle were monitored using a software-assisted (SCA20 software) image processing procedure. All the contact angle experiments were carried out in triplicate (n=3) at ambient conditions (22-40 % RH and 18 - 25.5 °C temperature).

2.2.11. Determination of surface energy parameters

Two liquids of known total (γ_l), dispersive (γ_l^d) and specific (γ_l^{SP}) surface free energy are enough to measure the total (γ_S), dispersive (γ_s^d) and specific (γ_s^{SP}) surface free energy of the solids using the following equations (Fowkes, 1964; Wu, 1973). The details of surface tension property of liquids used in this experiments are given in Table 1.

$$\gamma_l(1+cos\theta) = 2\left(\sqrt{\gamma_s^d\gamma_l^d} + \sqrt{\gamma_s^{SP}\gamma_l^{SP}}\right) \qquad \text{(Eq 4)}$$

where θ is the initial contact angle. Although surface energy parameters are normally calculated using equilibrium contact angle, exceptionally initial, or extrapolated initial, contact angle can also be used for hydrophilic surfaces where the contact angle changes with time (Correia, Ramos, Saramago, & Calado, 1997; Erbil, Yasar, Süzer, & Baysal, 1997; Shen, Sheng, & Parker, 1999; Adão, Saramago, & Fernandes, 1999; Ho, & Khew,. 2000; Luner, & Oh, 2001, Saurí, , et al., 2015).

If an apolar liquid (e.g., diiodomethane) is placed on the surface of a solid and its contact angle is measured, Eq. 4 can be reduced to Eq 5:

$$\gamma_l(1+cos\theta) = 2\sqrt{\gamma_s^d\gamma_L^d} \qquad \text{(Eq 5)}$$

As γ_l^{SP} of an apolar liquid is zero, when the γ_s^d and γ_s^{SP} of a solid are known, its γ_S can be calculated using (Eq 6):

$$\gamma_S = \gamma_s^d + \gamma_s^{SP} \quad \text{(Eq 6)}$$

The cohesion work of the solid W_c can be determined from the surface energy as per Eq 7:

$$W_c = 2\gamma_S \quad \text{(Eq 7)}$$

3. Results and discussion

3.1. Extraction and characterisation of okra biopolymer

The extraction protocol outlined in Fig.1 resulted in the isolation of different grades of okra biopolymer of varying purity (Table 2). It is reported that the temperature has a significant impact on the quantity and quality of isolated okra biopolymer (Samavati, 2013) so extraction of okra biopolymer from okra pods was performed at 70° C (± 2.5 °C). It was noted that the crude, purified and bi-purified protocols produced 17.21, 12.25 and 9.44 g (okra biopolymer (g) /100 g of dry okra pods), respectively, with yield decreasing as expected with relative purity. For each grade (crude, purified and bi-purified), drying was carried out at 40° C for 24 h and the powder remained visually homogeneous and without any colour changes. Although all grades were subjected to similar drying conditions for consistency, the water content of the products may therefore vary. Moreover it was evident from the results that the total sugar content increased and protein content decreased with relative purification steps (Table 2). The main components of okra biopolymer (i.e. galactose, rhamnose, and galacturonic acid) were qualitatively identified in the FTIR spectra for all the grades as shown in Fig. 2(a). A broad peak at 3280-3290 cm^{-1} in the spectrum, indicating the presence of aromatic sugar groups with O–H as the main functional group,

was found in the three samples of okra biopolymer. O–H groups are able to bind with water molecules and produce bound moisture within the polymer components. The existence of O–H groups confirms the hydrophilic characteristics within the polysaccharide. The medium peak that is visible at 2930-2942 cm^{-1} represents the C–H stretch that exists in galactose and rhamnose. The major functional groups are typically in the region between 1000 and 2000 cm^{-1} of the FTIR spectra. The carbonyl bands at 1722 cm^{-1} and 1601-1614 cm^{-1} indicate the esterified and free carboxyl groups, respectively. It is evident from Fig. 2a that, as the purification of okra biopolymer increased, the intensity of esterified carbonyl band enhanced attributing to higher esterification (Alba, Ritzoulis, Georgiadis, & Kontogiorgos, 2013; Nep, Sims, Morris, Kontogiorgos, & Smith, 2016). The identical small peak at 1415-1416 cm^{-1} indicates a C–H bend which is a constituent of galactose and rhamnose. The frequency of 1200–1000 cm^{-1} indicates C–O stretch bonds which are present in the aromatic compounds of galactose, rhamnose and galacturonic acid. The methyl, carbonyl, and hydroxyl functional groups that are present in the chemical structure of okra are constituents of the carbohydrate molecule, which is the main backbone of the polymer. More importantly it is clearly evident from Fig. 2a that the purification has no impact on the FTIR spectra of various grades of okra biopolymer. The XRD spectra Fig. 2b confirmed that the biopolymer consists of amorphous regions. The broad distribution that could be seen from the X-ray diffraction spectrum indicates the amorphous nature of the polymer. FTIR and XRD spectra of okra biopolymer confirm that the purification process has no effect on the molecular structural arrangement of okra biopolymer in general.

3.2. Solubility studies

The saturated solubility of different okra biopolymer grades was carried out over a wide pH range to investigate the impact of pH and purification on the solubility of okra biopolymer. It can be noted that the purification has a marked impact on the solubility of okra biopolymer. The crude grade has the lowest overall solubility at any given pH liquid followed by purified and bi-purified okra biopolymer (Fig 3a). This might be due to the presence of different small entities, depending on the pH, being produced during purification, thus impacting the performance of solubility measurements. Non-polymeric materials usually dissolve promptly, and the dissolution process is generally controlled by the external mass transfer confrontation through a liquid layer adjacent to the solid–liquid interface (Miller-Chou & Koenig, 2003). The solubility behaviour of okra biopolymer is complex in that solubility increases with increasing pH, which is likely due to ionisation phenomena. However, above pH 7.4, the solubility starts to reduce. An explanation is that at low pH (acidic conditions), the network of okra biopolymer chains is retained in a collapsed state due to very minimal ionisation of carboxyl groups (Berger, Reist, Mayer, Felt, Peppas, & Gurny, 2004), thus corresponding to low solubility of okra biopolymer in low pH liquids. However, as the pH of the liquid increases, the solubility of okra biopolymer increases until it reaches a maximum solubility at pH 7.4. This can be attributed to the higher/complete ionisation of carboxyl groups resulting in intra-ionic repulsion as the pH was increased further, the solubility falls due to dissociation of ionic bonds within the polymer molecular conformation that can potentially lead to breaching of their intact network (Berger et al., 2004; Kaur, Singh, & Brar, 2014).

3.3. Swelling and erosion studies

The swelling of a polymer is a function of rate and extent of chain relaxation. The rate of polymer swelling is an important aspect in controlling drug release from hydrophilic matrices. The swelling study of okra biopolymer matrices was primarily conducted to understand the liquid uptake and polymer-liquid interactions. The matrix tablets made of okra biopolymer (crude, purified or bi-purified) were immersed into the swelling liquids and their response is shown in Fig. 3b, in terms of the weight increased due to sorption versus time. The tactile and visual evaluation confirmed the development of a so-called gel on the surface of the compact. Regardless of the type of okra biopolymer, all the matrices were slippery to touch and swelling increased in the initial phase, however, it reduced in the later phases of the swelling study.

It is also apparent that the purification and pH of the swelling media have a perceptible impact on the liquid uptake behaviour. The swelling of crude okra biopolymer based matrix tablets was lowest in acidic (pH 1.2) rather than alkaline media (pH 7.4), Fig. 3b. It can also be seen that the bi-purified okra biopolymer matrices have a higher extent of swelling than other okra biopolymer grades, irrespective of swelling media.

Moreover, it can be hypothesised that the swelling behaviour of biopolymer based matrices, such as those observed in this study, occurs as a result of osmotic stress exerted at the moving front located between the dry glassy core and the outer gel layer. There are two transport processes, namely solvent diffusion and chain disentanglement involved in liquid imbibition into the matrix tablets, when it is in contact with a thermodynamically compatible liquid, the liquid will diffuse into the okra polymer chains. Due to plasticization of the polymer by the solvent, a gel-like swollen layer is formed (Ghori, 2014; Ghori & Conway, 2015). The swelling data was analysed using the Vergnaud liquid uptake

mathematical model (Vergnaud, 1993) to determine the rate and mechanism of swelling. The generalised form of Vergnaud model used is shown in Eq. 8

$$M = Kt^n \qquad \text{(Eq 8)}$$

Where,

M = the amount of liquid transferred

t = time

K = the swelling constant.

n = exponent indicating the mechanism of water uptake.

It is reported that a value of n < 0.5 is indicative of a diffusion-controlled mechanism in which the rate of diffusion is much slower than the rate of polymer hydration in a matrix tablet. However, when n = 1, water diffuses through the matrix at a constant velocity, with an advancing liquid front marking the limit of liquid penetration into the matrix. A value of 0.45 < n < 1 indicates an anomalous behaviour in which diffusion of liquid and polymer hydration are of similar magnitude (Ebube et al., 1997). The Vergnaud swelling kinetics parameters, enlisted in Table 3, depend on the porosity and the diffusivity of water in the matrix and are an indicator of polymer hydration. In general, the R^2 values were in the range of 0.986-0.999, which indicate that the data can be well described by this model. Bi-purified matrices have the highest swelling rate (K_w) 13.16 % min^{-1} and 9.59 % min^{-1} in pH 7.4 and pH 1.2 liquid media, respectively. Additionally, the crude okra biopolymer matrices have lowest K_w (3.16 % min^{-1} and 1.64 % min^{-1} in pH 7.4 and pH 1.2 liquid media, respectively). Thus purification has a significant impact on the swelling kinetics of matrices, moreover, the porosity of the matrices was also reduced from 30.2 % (crude biopolymer matrix tablet) to 18.5 % (bi-purified biopolymer matrix tablet) with an increasing level of

purification (Table 3). The correlation between porosity and swelling rate revealed that, with decreased porosity, the swelling rate has increased which might be attributed to the higher osmotic stress within the compact (Table 3), (Wise, 2000). As the bi-purified okra biopolymer has higher solubility, it can be hypothesised that the more soluble okra biopolymer matrices tend to develop micro-cavities influencing the osmotic stress and tortuosity of the matrix network, leading to a higher degree of swelling.

The swelling mechanism can be inferred from the swelling exponent (*n*), Table 3. According to the criteria laid out by Ebube et al., (1997), the purified matrix tablets exhibited diffusion controlled swelling where the rate of polymer relaxation is greater than the rate of liquid penetration into the polymer matrix network. However, all the other grades of okra polymer showed anomalous swelling behaviour in which the rate of water diffusion and polymer relaxation are of similar magnitude. The diffusion-oriented and increased rate of swelling in alkaline pH media can be attributed to ionisation of the carboxyl group within the okra biopolymer (Berger, Reist, Mayer, Felt, Peppas, & Gurny, 2004). Moreover, the ionisation of these groups increases the permeability of water molecules in the polymer network and the development of ionic bonds incorporating water molecules (Khare & Peppas, 1995). A schematic representation of this theory is illustrated in Fig. 4.

Once the hydrophilic matrices are hydrated, a gel layer develops along with two separate interfaces, one between the glassy polymer and gel layer and the other between the gel layer and the solvent. After an induction period, the polymer starts to dissolve, which is usually termed matrix erosion (Ghori, Ginting, Smith, & Conway, 2014). The erosion studies were carried out on all types of okra biopolymer matrices, in two different liquid media (pH 1.2 and pH 7.4) and the results are depicted in Fig. 3c. In due course, after the swelling

phase, the hydrophilic polymer-based matrices underwent matrix erosion. The rate of polymer erosion was determined using the data in Fig. 3c and the erosion kinetics parameters are enlisted in Table 3, with R^2 in the range of 0.958-0.988.The crude okra biopolymer matrices exhibited the fastest erosion rate ((K_E) 0.204 % min^{-1} and 0.265 % min^{-1} in pH 1.2 and pH 7.4 media, respectively). However, the bi-purified biopolymer based matrices has the slowest erosion [pH 1.2 (0.125 % min^{-1}) and pH 7.4 (0.145 % min^{-1})]. Fig. 3d shows a good correlation between matrix swelling and erosion, and indicates that higher swelling leads to low erosion. One explanation for this is a greater entanglement of polymer chains on the surface of matrix tablet in the purified grade and it is evident from Fig. 3d that the purification process has a marked effect on the relationship. Swelling of okra biopolymer matrices occurs when hydrogen bonds and ionic bonds maintain the integrity of the hydrophilic polysaccharide matrix during the course of connection with the liquid. Therefore, for any given material, when the hydrogen bonds are weak in any given media, matrix erosion may prevail. Moreover, it can be noticed that the porosity of bi-purified matrices is lower than that of the other matrices. Therefore, the higher degree of swelling (Fig. 3b, Table 3), higher sugar content, low porosity and possibly molecular weight alteration are intuitively expected to be associated with stronger gel layer development that decisively controls the erosion of okra polymer chains.

Thus, it is apparent that the purification has a noticeable impact on the swelling and erosion kinetics of okra biopolymer matrices and this can be used as supportive information during, for example, the developmental phase of scaffolds for tissue engineering, drug or food based emulsions, oromucosal and nasal formulations. Additionally, specific grades of okra

biopolymer with explicit swelling and erosion rate can be selected to develop controlled release pharmaceutical dosage forms.

3.4. Surface texture, contact angle and energetics studies

The values of three dimensional root mean square roughness (*Sq*) of okra biopolymer based matrix tablets were determined using atomic force microscopy. The 3D AFM images of matrix tablet surfaces can be seen in Fig. 5 (a-c). It can be seen that the purification has a noticeable effect on *Sq*. The matrices have lowest *Sq* (61.01 ± 5.44 nm) and highest *Sq* (358.1 ± 11.23 nm) for bi-purified and crude okra biopolymer matrices, respectively (Table 4) Moreover, it is apparent from the AFM findings that the average *Sq* of all the matrices was in the acceptable threshold confirming their good compressibility aptitude (Narayan & Hancock , 2003). Fig. 5d (1&2) shows the relationship between *Sq*, K_W and K_E, respectively. It is evident from the graphs that, with increased roughness, swelling rate is decreased and conversely the K_E values increase. Moreover, it is also apparent from Fig. 5e that with a reduction in porosity (crude > purified > bi-purified), the *Sq* is also reduced.

Fig. 6a shows the contact angle profile of okra biopolymers with water as the testing liquid. It was found that the contact angle of bi-purified matrix tablet was lower than other matrices. The water contact angle exponentially decreased as a function of time for all tested matrices. However, the diiodomethane contact angle showed a minor initial increase and then it became constant as a function of time (Fig. 6b). During the experiments with water as a test liquid, it was observed that drops placed on the bi-purified biopolymer matrices were immediately taken up by the compacts in accordance with its swelling behaviour. This may be due potentially lower moisture content (surface and in-bound) as the drying procedure may not produce consistent content across the grades. Moreover, this

may also be attributed to altered particle size distribution due to compression pressure. However, diiodomethane does not penetrate into the compact surface as quickly as water. These different responses of the compacts towards the liquid droplets reflect their hydrophilic nature and wettability. The wettability of the compact surface can be inferred using the contact angle criteria (Yuan & Lee, 2013). The wettability trend was crude < purified < bi-purified. Fig. 6c shows the calculated surface energy values of these matrices. A general decline in specific energy and surge in dispersive and total energy is evident with increasing sample purity therefore, it can be established that the purification can affect the surface energetics of the of okra biopolymer. Fig. 6d shows the work of cohesion (Wc, $mJ.m^{-2}$) of okra biopolymer matrices. According to Dupré, Wc is the work done *per* unit area produced in dividing a homogeneous liquid. The Wc specifies the work which must be expended to produce droplets from a volume of liquid when applied on the sample surface (Dupré & Dupré, 1869). When a liquid phase comes into contact with a second liquid or solid phase, the tendency to spread (complete wetting) is given by the ratio between the Wc per phase. It is apparent from the results, Fig. 6d, that the crude biopolymer based matrices have higher Wc while the bi-purified biopolymer based matrices have the lowest. The contact angle and surface energetics show that the purification of okra biopolymer has a measurable effect on the wettability and surface energetics of matrices. Moreover, these findings corroborate results from solubility studies. The most likely cause is the variation in the chemical composition of okra biopolymer, as the more purified samples have higher sugar content and are assumed to be more hydrophilic. Also, different distributions of ionisation sites within the okra polymer chains may impact the conformation of biopolymer chains on the surface of compact.

Furthermore, a semi-empirical approach has been introduced in order to describe the evolution of water contact angle (Farris et al., 2011) in biopolymer films. Using this current approach, we can are apply the same three parameter decay function model (Eq 10) to polymer matrix tablets (Eq 9 and Eq 10).

$$\theta\ (t) = \theta(0)\ exp\ (kt^n\ ) \qquad \text{(Eq 9)}$$

with its first derivative;

$$\frac{d\theta\ (t)}{dt} = kn\ \theta\ (t)\ t^{n-1} \qquad \text{(Eq 10)}$$

The mathematical function was first fitted to the experimental θ values collected during the 60 second periods of analysis with the goal of obtaining an adequate and simple analytical expression and its first derivative. The contact angle kinetic parameters are enlisted in Table 4. The contact angle experimental data fit acceptably well and the R^2 values were in the range of (0.996-0.998). Here, the *k* values for okra biopolymer matrices are -0.961, -1.172 and -1.279 for crude, purified and bi-purified grades, respectively. The *k* coefficient is the measure of contact angle evolution. Regarding the contact angle exponent (*n*), fractional values are normally attributed to the occurrence of two (or even more) simultaneous processes which are represented by absorption and spreading. Tentatively, *n* =0 and n=1 should be absolute absorption and spreading, respectively. So, according to these criteria, all the matrices exhibit absorption dominant wetting dynamics (Table 4). The validity of extending this concept to other polymer matrices will be further explored with different biopolymers and other excipients.

4. Summary and conclusions

The present study has shown that the purification processes led to a reduction in the protein; however the overall sugar content was increased with additional steps. Importantly, none of the purification processes was able to completely remove the protein contents. Therefore, it can be assumed that some protein fractions might be an integral part of the molecular structure of okra biopolymer. FTIR and XRD spectra of okra biopolymer confirmed that the purification process has no effect on the molecular structural arrangement of okra biopolymer, hence, confirming the suitability of extraction and purification protocol.

The different grades of okra behaved in a discrete way with respect to the surface interaction with liquid. They demonstrated different wetting kinetics and surface energetic properties as indicated by the contact angle analysis. The experimental confirmation herein suggests that the wetting trend was governed by the solid/liquid interface, namely, absorption which impacts the overall wetting dynamics.

The relative purity impacted solubility, and swelling and erosion behaviour of okra-based tablet matrices. The solubility and K_w were increased; however, K_E was reduced in any given liquid media. Information from the swelling and erosion studies provides understanding of the fundamental solvation behaviour of biopolymers and these findings will be helpful in the development and optimisation of end products. A deeper understanding of changes of protein fractions for different grades of refined okra might be a helpful next step.

AFM-based 3D surface texture analysis showed that bi-purified okra biopolymer has a smoother surface. Moreover, with the reduction in surface roughness, the swelling and surface energy was increased and the matrix erosion and porosity were reduced.

Hence, on the basis of these findings, it can be concluded that the present extraction and purification protocol of okra can be adopted to develop a plant-derived biopolymer having an acceptable level of purity. As the purification method can influence the way the biopolymer functions, thus specific needs for the formulation must be considered. More importantly, it is instinctively expected that the same can be true for analogous plant-derived polysaccharide biopolymers.

Acknowledgements

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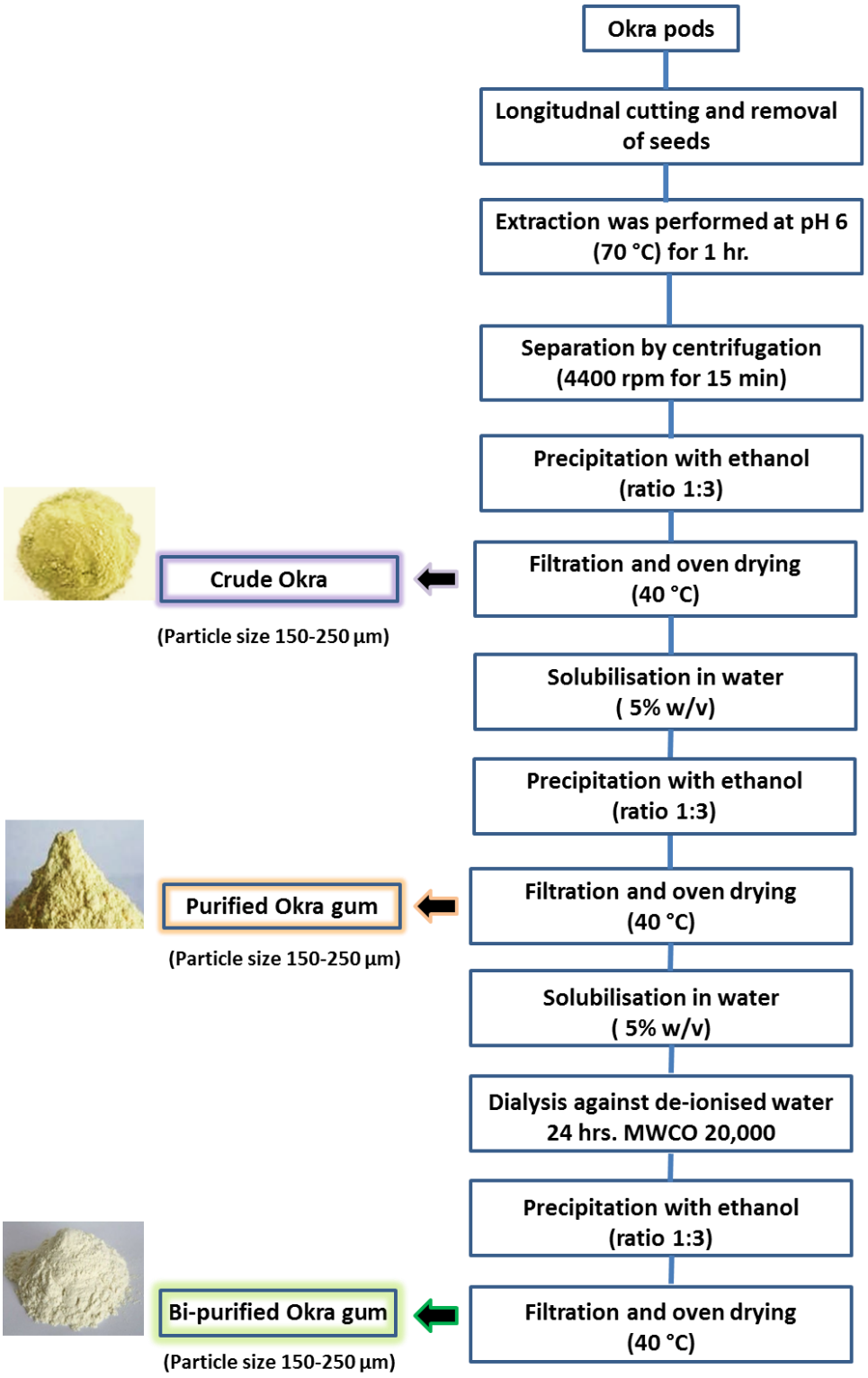

Fig. 1, Extraction and solubility protocol for okra biopolymer.

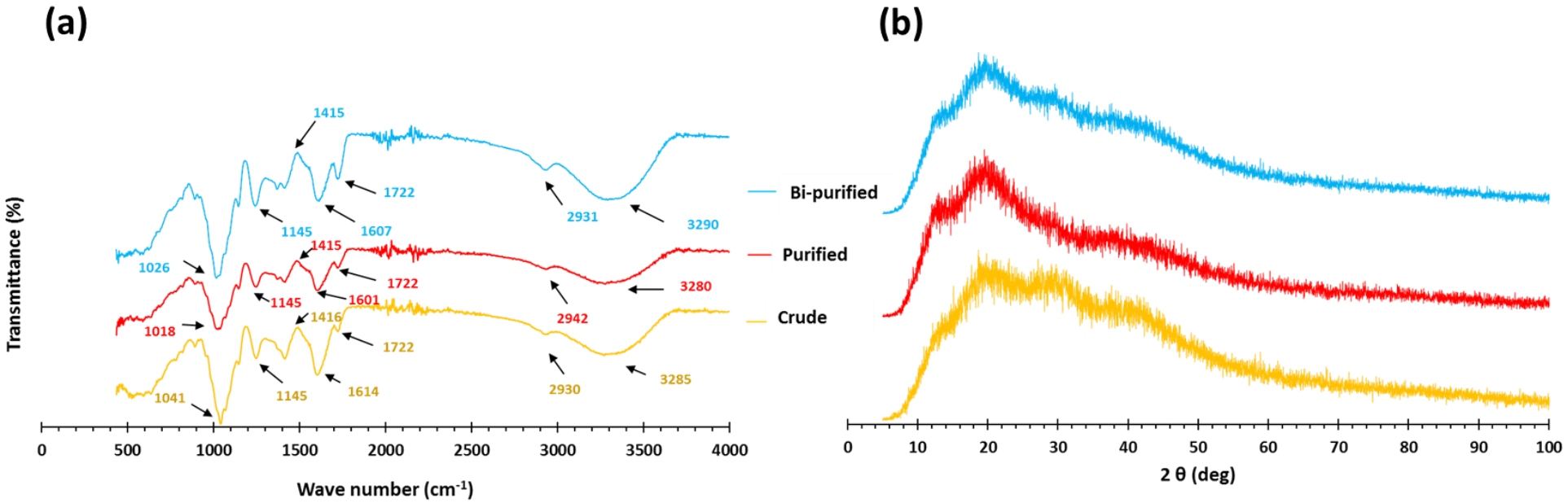

Fig. 2, (a) Fourier transform-infrared (FT-IR) and (b) X-ray diffraction (XRD) spectra of crude, purified and bi-purified okra biopolymer.

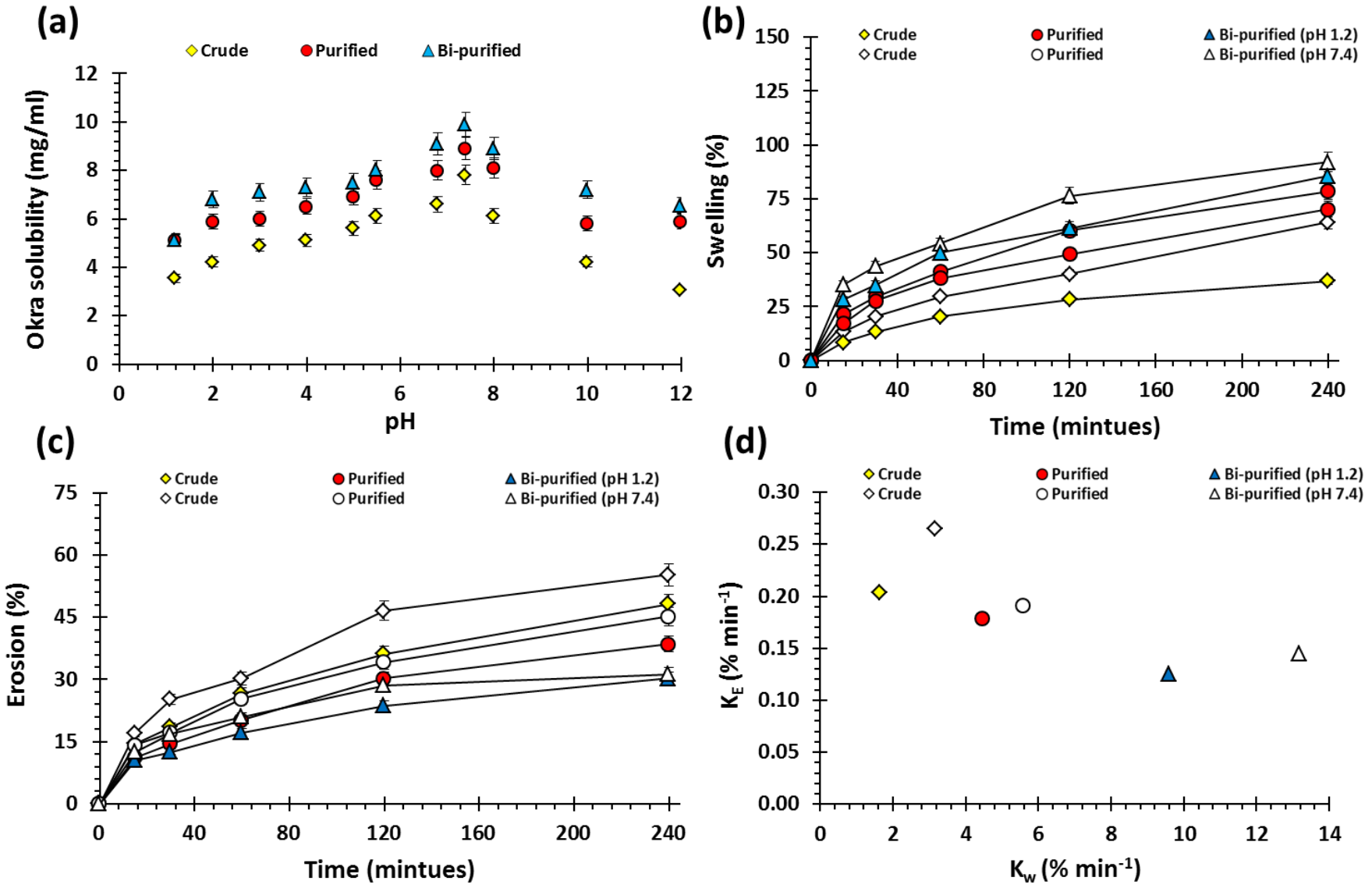

Fig. 3, (a) pH solubility profiles, (b) swelling, (c) matrix erosion (d) inter-relationship of swelling and erosion profiles of okra biopolymer based matrices.

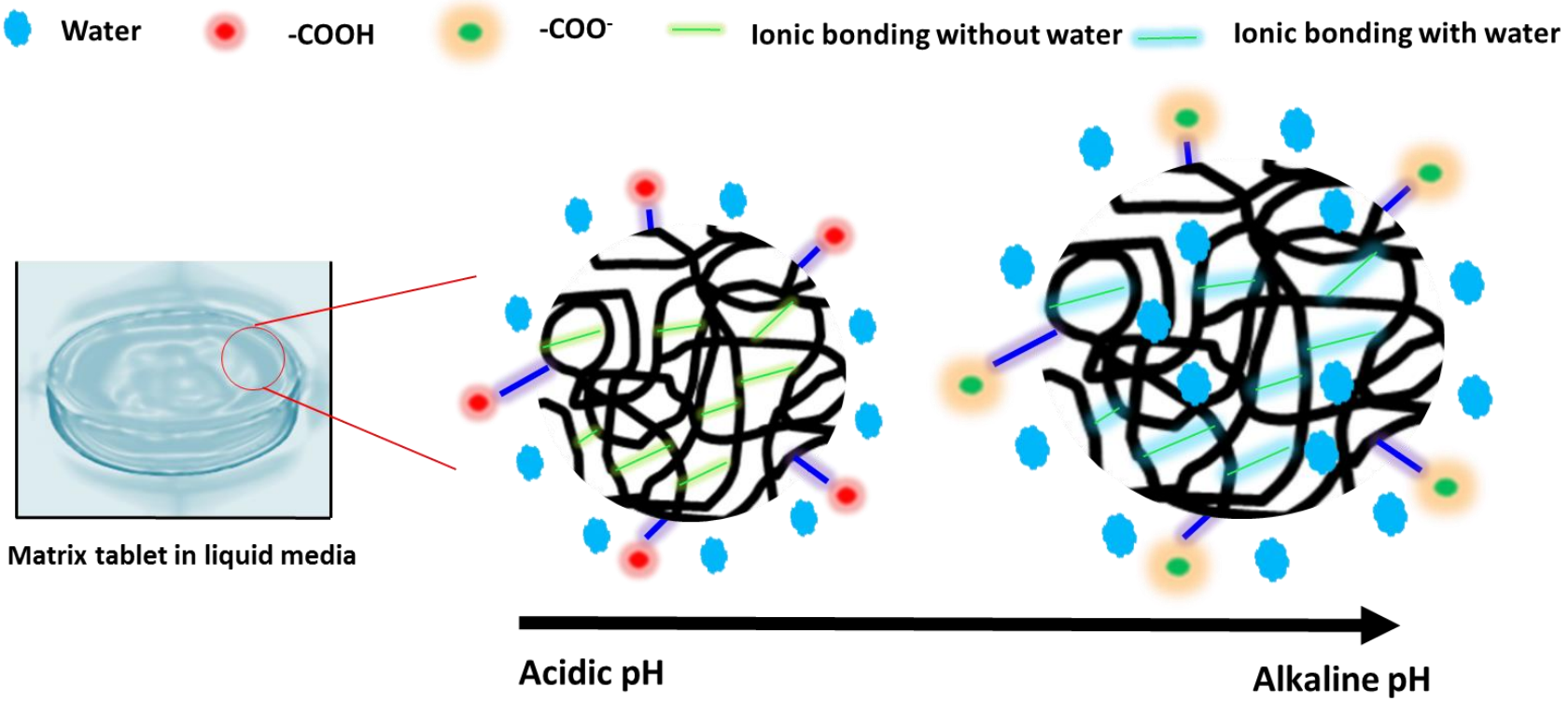

Fig. 4, Schematic illustration of okra biopolymer matrix tablet swelling process with respect to acidic and alkaline pH.

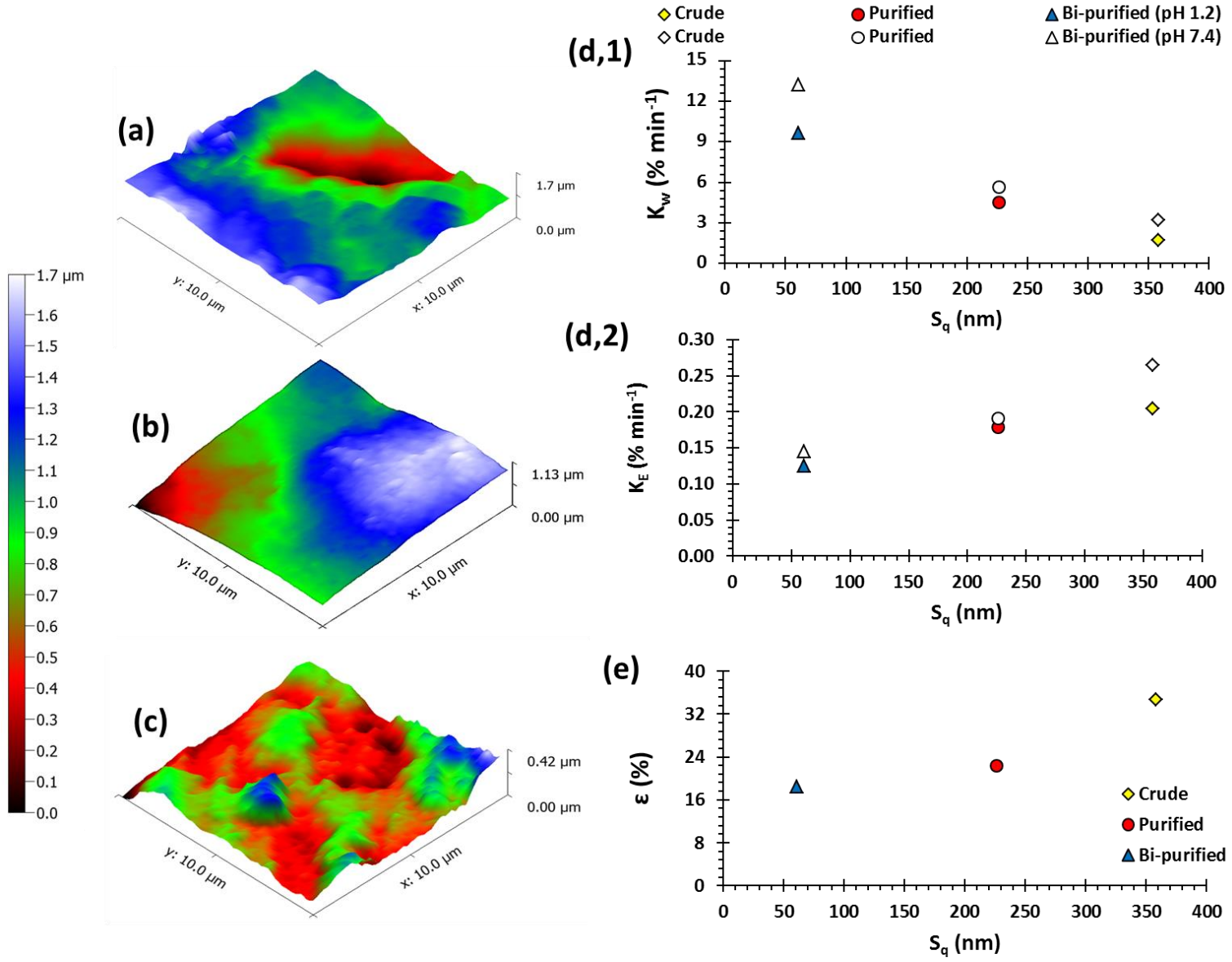

Fig. 5, Surface topography of (a) crude, (b) purified, (c) bi-purified and inter-relationship between surface roughness and swelling, and erosion of okra biopolymer matrices.

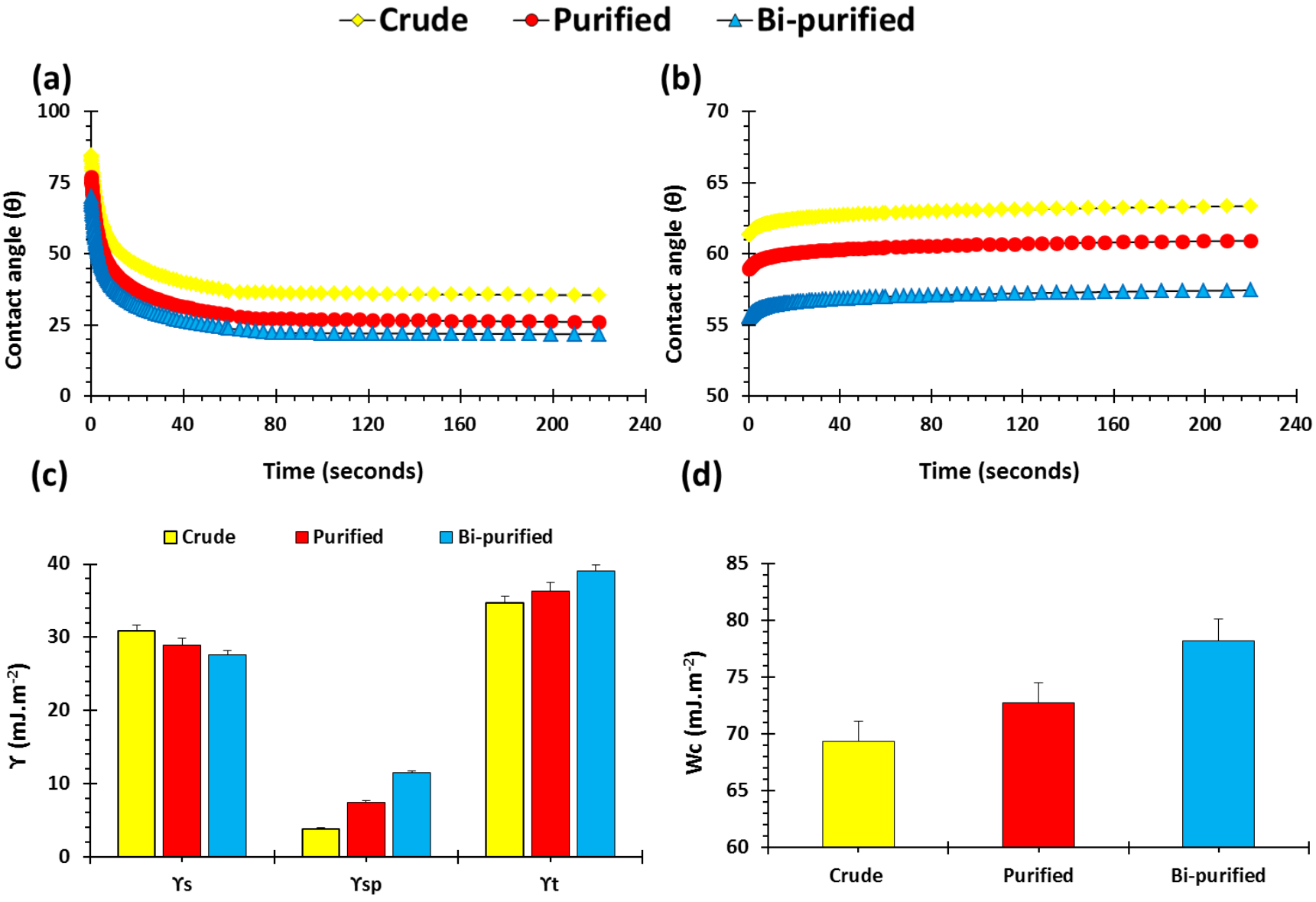

Fig. 6, Contact angle with (a) water, (b) diiodomethane, (c) surface energy and (d) work of cohesion profiles of okra biopolymer matrices.

Table 1, Total (γ_l), dispersive (γ_l^d) and specific (γ_l^{SP}) surface free energy of water and diiodomethane (Zajic and Buckton, 1990; Wu, 1971) .

Liquid	γ_l (mJ/m^2)	γ_l^d (mJ/m^2)	γ_l^{SP} (mJ/m^2)
Water	72.8	21.8	51.0
Diiodomethane	50.4	50.4	0.0

Table 2, Chemical composition of crude, purified and bi-purified okra gum (standard deviations are in parenthesis)

Okra gum grade	Total sugars (%)	Protein content (%)	Yield (g /100g okra pods)

Crude	68.55 (2.51)	7.14 (0.37)	17.21 (1.12) 49
Purified	77.33 (3.75)	5.55 (1.02)	12.25 (1.55)
Bi-purified	88.12 (3.41)	4.67 (0.51)	9.44 (0.87)

Table 3, Swelling and erosion kinetics, true density and porosity of matrix tablets

Okra gum matrices	pH of media	Swelling kinetics parameters			Matrix erosion parameters		ρ (gcm^{-3}) [d]	ε (%) [e]
		K_W [a]	n [b]	R^2	K_E [c]	R^2		
Crude	**1.2**	1.64	0.594	0.993	0.204	0.982	1.61 ± 0.01	30.2 ± 3.1
	7.4	3.16	0.530	0.995	0.265	0.980		
Purified	**1.2**	4.45	0.501	0.980	0.179	0.997	1.52 ± 0.02	24.7 ± 1.2
	7.4	5.58	0.496	0.999	0.191	0.981		
Bi-purified	**1.2**	9.59	0.387	0.989	0.125	0.992	1.56 ± 0.01	18.5 ± 2.7
	7.4	13.16	0.367	0.986	0.145	0.978		

[a] Swelling rate (% min^{-1}), [b] Swelling exponent, [c] Erosion rate (% min^{-1}), [d] True density, [e] Porosity

Table 4, Surface roughness and contact angle kinetic parameters

Okra gum	Sq (nm)	Contact angle kinetics parameters			
		ϑ	k	n	R^2
Crude	358.1 (11.23)	202.85	-0.961	0.143	0.996
Purified	226.5 (8.24)	223.95	-1.172	0.141	0.997
Bi-purified	61.01 (5.44)	225.67	-1.279	0.140	0.998