

1 **Nicotine stabilization in composite sodium alginate based wafers and**
2 **films for nicotine replacement therapy.**

3 Obinna C. Okeke, Joshua S. Boateng*

4 *Department of Pharmaceutical Chemical and Environmental Sciences, Faculty of*
5 *Engineering and Science, University of Greenwich at Medway, Central Avenue,*
6 *Chatham Maritime, ME4 4TB, Kent, UK.*

7 * Correspondence: Dr Joshua Boateng (J.S.Boateng@gre.ac.uk;
8 joshboat40@gmail.com)

9

10

11 **Abstract:**

12 Composite wafers and films comprising HPMC and sodium alginate (SA) were
13 formulated for nicotine (NIC) replacement therapy via the buccal route. Magnesium
14 aluminium silicate (MAS) was added in different concentration ratios (0.25, 0.5, 0.75)
15 to stabilize NIC and its effect on mechanical properties, internal and surface
16 morphology, physical form, thermal properties, swelling, mucoadhesion, drug content
17 and release behaviour of the formulations was investigated. MAS changed the physico-
18 mechanical properties of the composite formulations causing a decrease in mechanical
19 hardness, collapsed wafer pores, increased roughness of film surface, increase in
20 crystallinity and decreased mucoadhesion of the wafers. However, MAS increased
21 swelling in both films and wafers as well as interaction between NIC and SA, which
22 increased drug-loading capacity. Further, MAS resulted in rapid and slow release of
23 NIC from wafer and films respectively. The results suggest that the ideal formulation
24 for the stabilization of NIC in the composite formulations was MAS 0.25.

25
26 **Keywords:** Buccal delivery; Magnesium Aluminium Silicate (MAS); Nicotine; Nicotine
27 replacement therapy; Sodium alginate.

28
29 **1 Introduction**

30 Nicotine has been utilised as an active ingredient in the development of NIC
31 replacement therapy (NRT) via the oral mucosa (chewing gum, sublingual tablets,
32 lozenges), nasal mucosa (nasal spray and inhalers) and the skin (transdermal patch).
33 NIC liquid is volatile, alkaline and colourless with two well-separated pK_a values of
34 3.04 and 7.84, which can form diprotonated, mono-protonated and neutral NIC species
35 in an acid, neutral or basic solvent respectively (Pongjanyakul & Suksri, 2009). These
36 species can permeate membranes such as nasal, buccal and sublingual mucosae with

37 unionized species showing higher permeation than ionized forms (Nair, Chetty, Ho, &
38 Chien, 1997).

39 The oral mucosa of delivery has gained increased interest because of its ability
40 to avoid gastric acid, enzymes in the small intestine and first pass metabolism in the
41 liver, common with the conventional oral route (Sattar, Sayed, & Lane, 2014). The
42 buccal mucosa is highly vascular, less vulnerable to irritation and has a lower amount of
43 enzyme activities compared to intestinal, rectal, vaginal and nasal mucosae (Boateng &
44 Okeke, 2014). Though the use of the buccal mucosa for NIC delivery has been
45 demonstrated in NIC chewing gum, Nicorette®, a large percentage of the drug is
46 swallowed before achieving complete absorption (Nair et al., 1997; Adrian, Olin,
47 Dalhoff & Jacobsen, 2006; Benowitz, Jacob, & Savanapridi, 1987).

48 Alternative buccal delivery systems, which can be utilised in NRT using
49 mucoadhesive polymers have been under investigation including films (Aguzzi, Cerezo,
50 Viseras, & Caramella, 2007) and wafers (Aguzzi et al., 2007; Boateng & Areago, 2014)
51 and demonstrated improved functional properties when different polymers were
52 combined. Hydroxypropylmethylcellulose (HPMC) and sodium alginate (SA) have
53 been widely used as mucoadhesive polymers in the development of buccal-adhesive
54 drug delivery systems (Boateng & Areago, 2014; Manivannan, Balasubramaniam,
55 Anand, Sandeep, & Rajkumar, 2008; Adhikari, Nayak, Nayak, & Mohanty, 2010;
56 Pandey, Hingawe, Das, & Patil, 2014; Khan, Boateng, Mitchell, & Trivedi, 2015).
57 HPMC is a hydrophilic non-ionic semi-synthetic polymer widely used in the
58 pharmaceutical and food industries while SA is a poly-anionic polysaccharide polymer
59 made up of alginic acid (a polyuronic acid composed of mannuronic and guluronic acid
60 residues), extracted from brown seaweed. HPMC-SA composites were reported for the

61 formulation of buccal NIC tablets for smoking cessation (İkinci, Şenel, Wilson, &
62 Şumnu, 2004).

63 The challenges posed by NIC are its volatility and oxidative degradation of the
64 free base. To address these challenges, there has been research into the adsorption of
65 NIC onto several materials such as cellulose powder (Mihrianyan, Andersson, & Ek,
66 2004), cation exchange resins (Rakić et al., 2010) and inorganic clays such as
67 magnesium aluminium silicate (MAS) (Pongjanyakul & Suksri, 2009). In particular,
68 polymer-clay composites having improved mechanical properties, thermal behaviour
69 and modified drug release have attracted interest in the field of drug delivery (Aguzzi et
70 al., 2007; Gilman, 1999; Pavlidou & Papaspyrides, 2008).

71 MAS results from the combination of natural smectites (montmorillonite and
72 saponite clays) that forms a layered structure (Rowe, Sheskey, & Owen, 2006;
73 Pongjanyakul & Suksri, 2009), comprising three-lattice layers of octahedral alumina or
74 magnesia and two tetrahedral silica. Upon hydration, the MAS layered structure
75 separates, exposing the weakly positively charged edges and negatively charged faces.
76 This can readily interact with amine drugs such as NIC, as well as demonstrate
77 electrostatic interaction, which contributes to slow drug release in formulations
78 (Pongjanyakul & Suksri, 2009; Rowe et al., 2006). MAS incorporated into NIC loaded
79 single polymer (SA) based films demonstrated interaction of MAS with anionic SA
80 polymer as well as increase in NIC retention within the films (Pongjanyakul & Suksri,
81 2010).

82 In this study, composite SA based films and wafers containing different
83 concentrations of MAS, loaded with NIC were characterised and compared for the first
84 time. The hypothesis is that the presence of SA and MAS within a composite

85 formulation will stabilize NIC and result in high drug loading suitable for NRT via the
86 buccal mucosa.

87

88 **2 Materials and methods**

89 **2.1 Materials**

90 Hydroxypropylmethylcellulose - HPMC (Methocel K100 premium LV) and
91 Magnesium aluminium silicate (MAS) were gifts from Colorcon Limited (Dartford,
92 UK) and R.T. Vanderbilt Company Inc (Norwalk, CT, USA) respectively. Sodium
93 hydroxide, potassium dihydrogen phosphate, gelatine were purchased from Fluka
94 Analytical (Buchs, Switzerland). Nicotine (liquid form), sodium alginate –SA
95 (molecular weight 120,000 – 190,000 g/mol, mannuronate/guluronate ratio 1.56), and
96 mucin from porcine stomach were all obtained from Sigma Aldrich (Dorset, UK);
97 sodium acetate, trimethylamine and glycerol were purchased from Fisher Scientific
98 (Loughborough, UK).

99 **2.2 Preparation of composite films**

100 NIC loaded MAS films were prepared in different ratios with a total polymer (HPMC-
101 SA) concentration of 2% w/v. The concentrations of polymers, MAS, plasticizer and
102 drug used in each polymer solution have been summarised in Table 1a. The polymeric
103 solutions for film formulation were prepared by dissolving glycerol (GLY) in 80ml of
104 distilled water while stirring at of 25°C before gradually adding HPMC and SA powder
105 one after the other and stirred between 500-700rpm for 2 hours. MAS on the other hand
106 was dissolved in 20ml of hot distilled water (50°C) for 30 mins, and mixed with the
107 dispersed polymeric solution. The resulting final solutions were left overnight (16-20
108 hrs) to eliminate air bubbles, NIC added to the MAS composite mixture and stirred at

109 low rpm (100-200rpm) for 30 mins. 30 g of the NIC loaded MAS solutions were poured
110 into a Petri dish (90mm diameter) and dried in an oven at 30°C for 18-20 hrs.

111 Table 1: (a) Composition of selected polymer, plasticizer, MAS and NIC used in
112 composite gel for film formulation and (b) Composition of selected polymers, MAS and
113 NIC used in composite gels for formulating wafers.

114 **(a) Films**

Sample name	HPMC	SA	GLY	MAS	NIC
	(% w/v)	(% w/v)	(% w/v)	(% w/v)	(g)
MAS 0.00	1.25	0.75	2.00	0.00	0.20
MAS 0.25	1.25	0.75	2.00	0.25	0.20
MAS 0.50	1.25	0.75	2.00	0.50	0.20
MAS 0.75	1.25	0.75	2.00	0.75	0.20

115 **(b) Wafers**

Sample name	HPMC	SA	MAS	NIC
	(% w/v)	(% w/v)	(% w/v)	(g)
MAS 0.00	1.25	0.75	0.00	0.20
MAS 0.25	1.25	0.75	0.25	0.20
MAS 0.50	1.25	0.75	0.50	0.20
MAS 0.75	1.25	0.75	0.75	0.20

116 **2.3 Preparation of composite wafers**

117 NIC loaded HPMC-SA-MAS solutions were prepared in a similar manner to films but
118 without using GLY. The solutions (1g) were poured into each well of a 24 well plate

119 (diameter 15.5mm). The concentrations of polymers, MAS and drug present in each
120 solution are summarised in Table 1b. The freeze-dried wafers were prepared using an
121 automated lyophilisation cycle, Virtis Advantage XL 70 freeze-dryer (Biopharma
122 process systems, Winchester, UK). The well plates containing the gels were loaded onto
123 the shelves of the freeze-dryer and programmed for freezing, primary drying and
124 secondary drying steps. The freezing step involved cooling the sample from room
125 temperature to 5°C (40 mins), 5°C to -10°C (40 mins), and then from -10°C to -55°C
126 (120 mins). An annealing step was incorporated into the freezing cycle by increasing
127 the temperature from -55°C to -35°C (2 hrs) and then cooling back down to -55°C (3
128 hrs). Additional freezing was performed at -55°C (1 hr) with a condenser temperature of
129 -55°C under pressure (200mTorr). The primary drying occurred under high pressure of
130 50mTorr. The temperature was raised from -55°C to -20°C (8 hrs) and further increased
131 from -20°C to -15°C ° (10 hrs). Secondary drying occurred at 50mTorr, from -15°C to
132 25°C (12.5 hrs).

133 **2.4 Polymer solution properties**

134 The polymeric solutions were analysed for surface stickiness, stringiness and gel
135 strength using a texture analyser (HD plus, Stable Micro System, Surrey, UK) equipped
136 with a 5 kg load cell. A 25mm probe was lowered onto the solution at a speed of
137 1mm/sec, held for 2 sec, and then withdrawn at a speed of 8mm/sec. The maximum
138 force at withdrawal of probe from sample was recorded as surface stickiness while the
139 distance from the onset and offset of force while moving the probe away from the
140 sample was recorded as stringiness. The viscous 'gel' strength was recorded as the
141 maximum force as the probe penetrated the polymeric solution to the required depth.

142 **2.5 Mechanical characterization using texture analysis (TA)**

143 **2.5.1 Tensile properties of films**

144 The tensile properties of the films were analysed using a texture analyser (HD plus,
145 Stable Micro System, Surrey, UK) equipped with a 5 kg load cell. The films (dumb-bell
146 shaped) were fixed between two tensile grips of the TA instrument and then stretched at
147 a test speed of 2mm/sec till breaking point. The elongation at break (%), tensile strength
148 and elastic modulus were determined ($n=3$) (Morales & McConville, 2011).

149 **2.5.2 Mechanical properties of wafers (hardness)**

150 The resistance to compressive deformation (hardness) of the freeze dried wafers was
151 determined using a texture analyser (HD plus, Stable Micro System, Surrey, UK)
152 equipped with a 5 kg load cell. The wafers were compressed to a depth of 2mm using a
153 2mm cylinder stainless steel probe in compression mode at a speed of 1mm/sec. Wafers
154 were compressed on 5 different sides ($n=3$).

155 **2.6 Scanning electron microscopy (SEM)**

156 The surface morphology of films and wafers were analysed using a Hitachi SU8030
157 (Hitachi High-Technologies, Krefeld, Germany) scanning electron microscope.
158 Formulations were cut and placed on an Agar Scientific G301 aluminium pin-type
159 stubs, using an Agar Scientific G3347N double-sided adhesive carbon tape. The films
160 were carbon coated, while wafers were gold coated using a Sputter Coater (Edwards
161 188 Sputter Coater S1508). The films and wafers were analysed at 2.0kV and 5.0kV
162 accelerating voltage respectively.

163 2.7 *Wafer porosity*

164 Pore analysis was performed in order to evaluate the porosity of wafer structure. The
165 wafers were initially weighed and then immersed in 5ml of ethanol in a glass vial and
166 left to stand for 10 mins to allow complete saturation with ethanol. The vials with
167 ethanol and wafers were degassed to remove air bubbles entrapped in the wafers for 10
168 mins. The wafers were carefully removed from the solvent, gently wiped to remove
169 excess solvent, and immediately weighed, to minimise loss of ethanol.

170 The percentage porosity of wafers was calculated using equation 1 below:

$$171 \quad P = \frac{V_p}{V_g} \times 100 = \frac{W_f - W_i}{\rho_e V_g} \quad (1)$$

172 Where

173 V_p = pore volume

174 V_g = wafers geometrical volume

175 W_f = final wafer weight

176 W_i = initial wafer weight

177 ρ_e = ethanol density (0.789 g/cm³)

178 2.8 *X-ray diffraction (XRD)*

179 The physical (crystalline/amorphous) form of NIC loaded MAS films and wafers was
180 investigated using a D8 Advantage X-ray diffractometer. Films were cut into small
181 pieces whilst wafers were compressed, placed on the holder and mounted onto the
182 sample cell. For pure starting materials, mylar was used to hold the powders before
183 placing on the sample cell. The samples were analysed in transmission mode at a
184 diffraction angle ranging from 5° to 50° 2 θ , step size 0.04°, and scan speed of 0.4s/step.

185 **2.9 Attenuated total reflectance Fourier transform infrared spectroscopy**
186 **(ATR-FTIR)**

187 ATR-FTIR spectra were obtained from a Perkin Elmer Spectrum instrument equipped
188 with a diamond universal ATR-unit. Strips of films and wafers and polymer powders
189 were separately placed on the ATR diamond crystal and force applied using a pressure
190 clamp to allow adequate contact between the sample and diamond crystal. NIC required
191 no force application as the liquid could form intimate contact with the diamond crystal.
192 The resolutions of the samples were recorded at 4 cm^{-1} within the range of 450-4000
193 cm^{-1} . Background spectra were subtracted in order to obtain a reliable absorbance of
194 each sample.

195 **2.10 Swelling**

196 The swelling capacities of films and wafers were determined by immersing each
197 formulation into 5ml of phosphate buffered saline (pH 6.8; ionic strength, 0.07M) and
198 change in weight recorded at time intervals of 2 mins up to 30 mins. For every time
199 interval, the medium was carefully removed to obtain an accurate weight of the sample
200 and replaced with fresh medium. Three replicates were performed for each sample and
201 swelling index (%) was calculated using equation 2 (Nair et al., 2013).

202
$$\text{Swelling index} = \frac{W_s - W_d}{W_d} \times 100 \quad (2)$$

203 Where W_d = dry weight of film or wafer.

204 W_s = weight of film or wafer after swelling.

205 **2.11 Mucoadhesion**

206 Adhesion test was performed on films and wafers using a TA. HD *plus* texture analyser
207 (Stable micro systems, Surry, UK) in tensile mode and fitted with a 5kg load cell. Films

208 were cut to match the mathematical area of wafers (a circle with diameter = 15.5mm).
209 The formulations were attached to an adhesive probe (75mm diameter) of the TA
210 instrument using a double-sided adhesive tape. Gelatine solution (6.67% (w/v))
211 prepared at 70°C (stirred at 500-700rpm) was poured into a Petri dish (86mm diameter)
212 and immediately placed in a fridge overnight (16-20 hrs) to set into solid gel, and 0.5 ml
213 of mucin solution (2% (w/v)) prepared in phosphate buffered saline (pH 6.8; ionic
214 strength, 0.07M) at room temperature was evenly spread on the gelatine gel to represent
215 the buccal mucosa. The probe with formulation attached was lowered to make contact
216 with the model buccal mucosa surface for 60 sec, at an applied force of 1.00N, and then
217 detached. Mucoadhesive strength was determined by the peak adhesive force (PAF)
218 required to detach the sample from the gelatine surface, total work of adhesion (TWA)
219 was determined by the area under the force-distance curve, while cohesiveness
220 represents the distance the samples travelled till they detached from the model buccal
221 surface. Texture Exponent 32[®] software was used in collecting and processing the data
222 from the TA analyser.

223 ***2.12 High performance liquid chromatography (HPLC)***

224 NIC was analysed by HPLC using an Agilent 1200 HPLC instrument (Agilent
225 Technologies, Cheshire, UK) with an auto sampler. The column used was a C-18
226 reverse-phase column, 4.6 x 250mm (Phenomenex, Cheshire, UK). Trimethylamine,
227 methanol and sodium acetate (88:12:0.5 v/v) were used as mobile phase and pH
228 adjusted to 4.2 using glacial acetic acid. Mobile phase flow rate was 1ml/min and
229 wavelength detection was set at 259nm (Pongjanyakul & Suksri, 2010). The retention
230 time of NIC was detected at approximately 4.5 min. Calibration curve was plotted using
231 standards with NIC concentration ranging from 40µg/ml to 400µg/ml ($R^2=0.9994$).

232 **2.13 Drug content**

233 The content of NIC in NIC loaded MAS films and wafers was assayed by accurately
234 weighing and dissolving films and wafers in 10ml of distilled water. The films and
235 wafers were accurately weighed (20-40mg) and recorded in determining the drug
236 content. The resulting solution was collected into a syringe, filtered through a 0.45µm
237 cellulose acetate membrane, transferred into HPLC vials and placed in HPLC sample
238 chamber and analysed as described above ($n=3$).

239 **2.14 In vitro drug dissolution**

240 *In vitro* drug dissolution of NIC loaded films and wafers was performed using a Franz-
241 diffusion cell apparatus. The receptor compartment was filled with 8ml of phosphate
242 buffer (pH 6.8) with a mesh (1mm mesh size) on the receptor surface. The donor and
243 receptor compartments were sealed with paraffin to limit evaporation and held together
244 by a pinch clamp. The system was placed on a water bath at 37°C with magnetic stirring
245 at approximately 200rpm. Formulations were accurately cut, weighed (20-40 mg) and
246 placed on the mesh between the donor and receptor compartments. At predetermined
247 time intervals, 0.5ml aliquots of the dissolution media were withdrawn using a 1ml
248 syringe, filtered through a 0.45µm cellulose acetate membrane, transferred into HPLC
249 vials and analysed using HPLC. The aliquot withdrawn was always replaced with fresh
250 buffer solution at 37°C. The percentage cumulative drug released from both films and
251 wafers were calculated and plotted against time ($n=3$).

252 Experimental release data was fitted to various kinetic models using
253 representative plots. These plot profiles include: cumulative % drug release vs time
254 (zero order kinetic model); log cumulative of % drug remaining vs time (first order
255 kinetic model); cumulative % drug release vs square root of time (Higuchi model); cube
256 root of drug % remaining in matrix vs time (Hixson-Crowell cube root law); and log

257 cumulative % drug release vs log time (Korsmeyer-Peppas model). (Dash, Murthy,
258 Nath, & Chowdhury, 2010; Singhvi & Singh, 2011).

259 **2.15 Statistical analysis**

260 The results were expressed as mean (\pm standard deviation) and statistical analysis was
261 performed using student t-test and / or one-way ANOVA to compare results. The
262 significant differences of data were determined at a level of $p < 0.05$.

263 **3 Results**

264 **3.1 Polymer solution properties**

265 The pH of the HPMC-SA solutions was neutral but increased to between pH 9-10 upon
266 addition of NIC. NIC loaded HPMC-SA-MAS solutions were less viscous and
267 therefore flowed easily when poured into both the well plates and Petri-dishes for
268 wafers and films respectively.

269 Table 2: Surface stickiness, stringiness and gel strength of HPMC-SA-MAS gel
270 formulations

<i>Formulations</i>	<i>Surface stickiness (g)</i>	<i>Stringiness (mm)</i>	<i>Gel strength (g)</i>
<i>MAS 0.00</i>	15.51 \pm 9.30	0.80 \pm 0.27	804.42 \pm 268.81
<i>MAS 0.25</i>	18.98 \pm 1.64	0.88 \pm 0.08	981.45 \pm 111.59
<i>MAS 0.50</i>	4.15 \pm 0.39	0.53 \pm 0.07	184.09 \pm 10.30
<i>MAS 0.75</i>	20.91 \pm 0.708	0.85 \pm 0.05	541.51 \pm 153.24

271
272 The HPMC-SA-MAS solutions (Table 2) also demonstrated increase in surface
273 stickiness, stringiness and 'gel' strength with initial increase in MAS concentration

274 from MAS 0.00 to MAS 0.25, but a decrease in stickiness, stringiness and gel strength
275 for MAS 0.50 formulation and a subsequent increase in stickiness, stringiness and ‘gel’
276 strength for the MAS 0.75 formulation. Overall, the MAS 0.25 formulation
277 demonstrated the highest value of stringiness and ‘gel’ strength compared to other
278 formulations, while MAS 0.75 formulation demonstrated the highest value of surface
279 stickiness. However, MAS 0.50 formulation demonstrated the lowest value of
280 stickiness, stringiness and ‘gel’ strength compared to the other MAS loaded
281 formulations. NIC loaded solutions were transparent with light brown colour but
282 transparency decreased as MAS concentration increased.

283

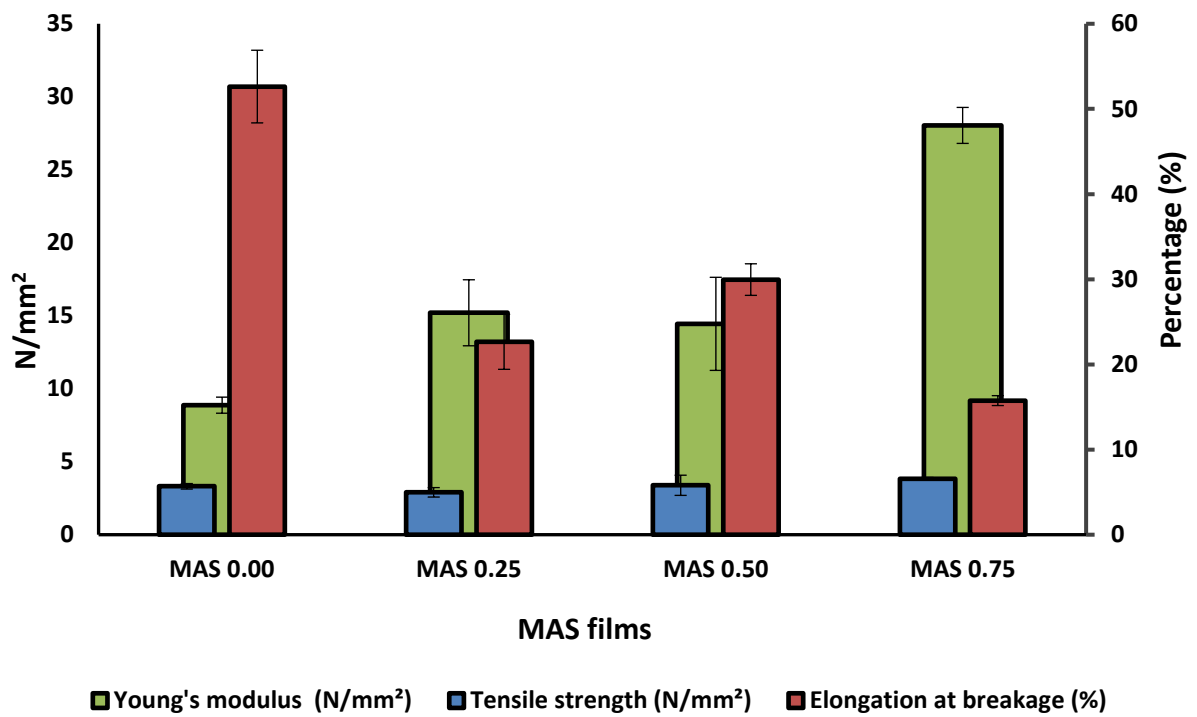
284 **3.2 Texture analysis (TA)**

285 **3.2.1 Tensile properties of films**

286 Figure 1a shows the tensile profiles of NIC loaded SA based composite films at
287 different MAS concentrations. The tensile strength of NIC loaded SA based composite
288 films ranged from $4.98 \pm 0.55\text{N/mm}$ to $6.58 \pm 0.15\text{N/mm}$. There was a gradual increase
289 in tensile strength as the concentration of MAS increased. Films with the lowest
290 concentration of MAS (0.25) showed the lowest tensile strength ($4.98 \pm 0.55\text{ N/mm}$)
291 while those with the maximum MAS concentration (0.75) showed the highest tensile
292 strength ($6.58 \pm 0.15\text{N/mm}$). There was also a significant difference ($p<0.05$) between
293 MAS 0.25 and MAS 0.75 tensile strength. A gradual increase in elastic modulus was
294 also observed as MAS concentration increased with the highest concentration of MAS
295 (MAS 0.75) exhibiting the highest value ($28.04 \pm 1.2327\text{N/mm}^2$) of elastic modulus. A
296 decrease in elongation at break (%) was observed as MAS concentration increased
297 which was most pronounced at the highest concentration of MAS (MAS 0.75) with a
298 value of $16 \pm 0.58\%$. Composite films with no MAS demonstrated the highest

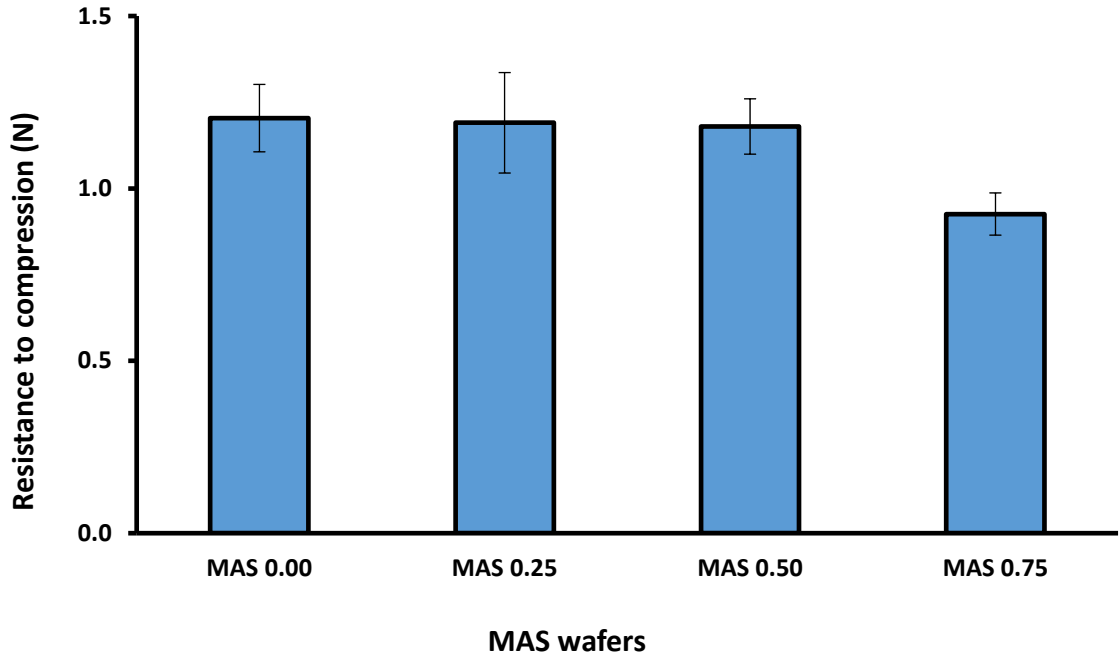
299 elongation at break (%) of 53 ± 4.27 followed by MAS 0.50 (30 ± 1.85). In general, the
300 concentration of MAS had an effect on the mechanical properties of NIC loaded
301 composite films.

302
303



304
305

(a)



306

307 (b)

308 **Figure 1** (a) Tensile properties of NIC loaded films ($n = 3$) and (b) Hardness profiles showing
 309 the resistance of NIC loaded wafers ($n = 3$) to compressive deformation forces.

310 3.2.2 Mechanical properties of wafer (hardness)

311 Figure 1b shows the hardness profiles of NIC loaded SA based composite wafers at
 312 different MAS concentrations. The results showed similar hardness values of $1.20 \pm$
 313 0.10 , 1.19 ± 0.15 and 1.18 ± 0.08 N for MAS 0.00, 0.25 and 0.50 wafers respectively,
 314 but decreased (0.93 ± 0.06 N) for wafers containing the highest amounts of MAS (0.75).

315 The results show that increase in the concentration of MAS up to MAS 0.50 did not
 316 affect the resistance of wafer to compression deformation force until the concentration
 317 exceeded MAS 0.50 (i.e. MAS 0.75) as demonstrated in Figure 1b.

318

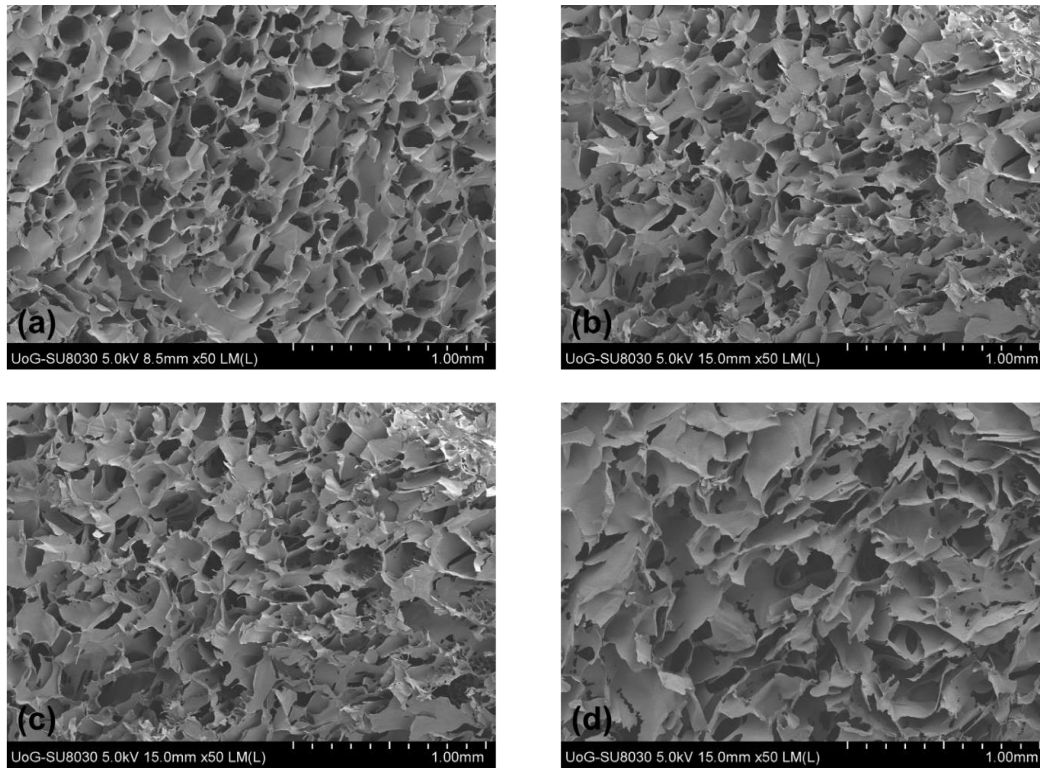
319 3.3 Scanning electron microscopy (SEM)

320 The internal structures and surface morphology of wafers and films, are shown in

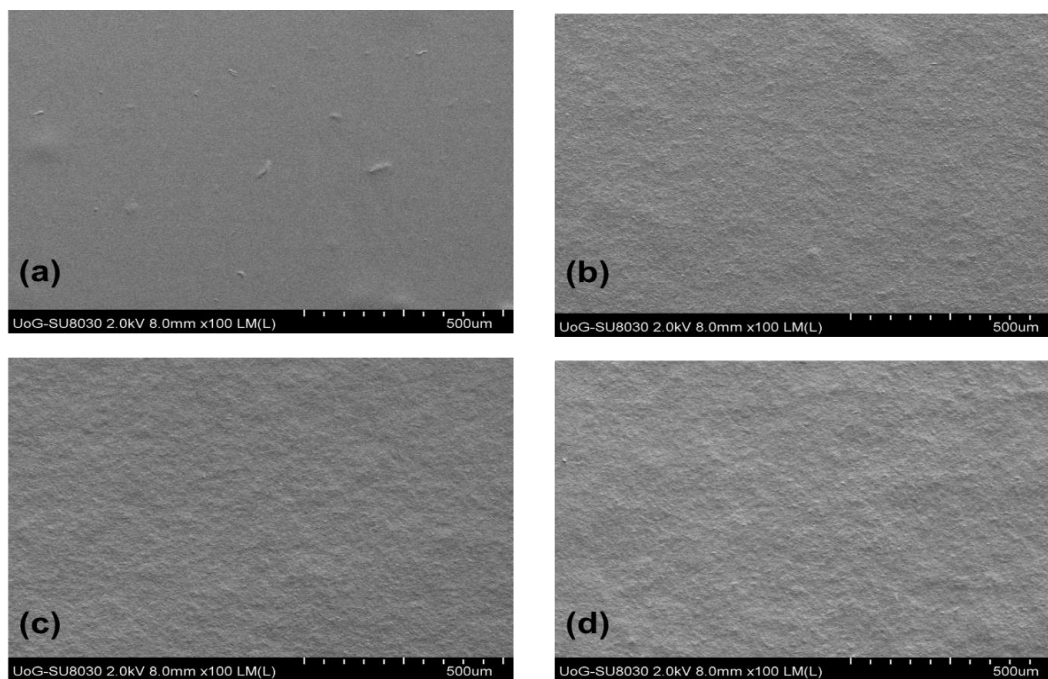
321 Figures 2 and 3 respectively. Wafers demonstrated a sponge-like and porous internal

322 structure while the films showed a continuous polymer sheet. The wafers showed

323 collapsed pore walls as MAS concentration increased with a highly collapsed wall
324 observed at MAS 0.75. The films also demonstrated a rough surface morphology as
325 MAS concentration increased with MAS 0.75 film showing the most uneven surface
326 compared to other films.



327
328 **Figure 2** SEM images of NIC loaded wafers containing different amounts of MAS: (a) MAS
329 0.00 (b) MAS 0.25 (c) MAS 0.50 and (d) MAS 0.75.



330

331 **Figure 3** SEM images of NIC loaded films containing different amounts of MAS: (a) MAS 0.00
 332 (b) MAS 0.25 (c) MAS 0.50 and (d) MAS 0.75.

333

334 3.4 *Wafers porosity*

335 Figure S1 (supplementary data) shows the porosity (%) of SA based composite wafers
 336 at different MAS concentrations. The results demonstrated a decrease in porosity as
 337 MAS concentration in the formulation increased from MAS 0.00 to 0.50, but showed a
 338 sudden increase at maximum MAS concentration (MAS 0.75). However, this cannot be
 339 conclusive because of the degree of error observed between MAS 0.50 and 0.75.

340 Generally, the result supports SEM results wafers with a better pore structure and
 341 homogeneity observed for HPMC-SA wafer with no MAS present (i.e. MAS 0.00).

342 3.5 *XRD analysis*

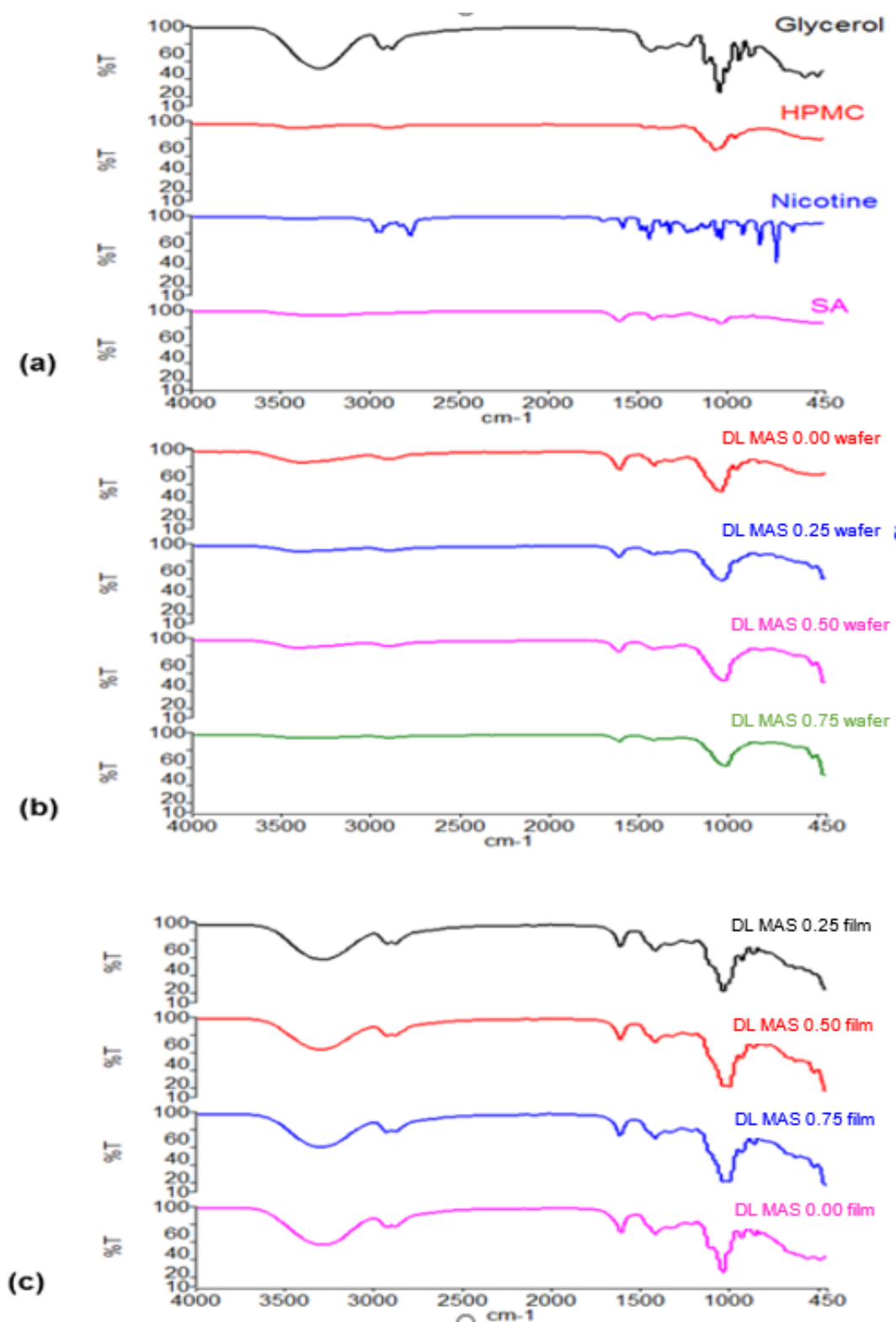
343 Figure S2(a) shows XRD transmission diffractograms of pure SA, HPMC, MAS and
 344 mylar (Okeke and Boateng, 2016). HPMC and SA demonstrated a broad peak at 2θ
 345 between $15^\circ - 24^\circ$ and $20^\circ - 23^\circ$ respectively suggesting amorphous structure. Unlike

346 HPMC and SA powders, MAS demonstrated a crystalline form with diffraction peaks at
347 2θ values of 20° , 22° , 23° and 29° , and a broad amorphous peak from 2θ of $34^\circ - 38^\circ$.
348 Figure S2(b) showed one crystalline peak at 2θ 23° in NIC loaded composite wafer
349 without MAS (MAS 0.00) but showed three crystalline peaks at 20° , 22° , 23° for all
350 other MAS formulations (i.e. MAS 0.25, 0.50 and 0.75), attributed to the presence of
351 MAS. NIC loaded wafer also demonstrated a broad peak from 2θ $15-24^\circ$ and from 2θ
352 $34^\circ - 38^\circ$. NIC loaded film without MAS showed a broad peak from 2θ $15-24^\circ$ while
353 MAS loaded films (i.e. MAS 0.25, 0.50 and 0.75) showed broad peaks from $15-24^\circ$
354 with two crystalline shoulders at 2θ of 20° and 22° .

355

356 **3.6 ATR-FTIR spectroscopy**

357 ATR-FTIR spectra of SA, HPMC), GLY, NIC, MAS, NIC loaded composite wafers and
358 films are shown in Figure 4.



359

360 **Figure 4** ATR-FTIR spectra of (a) pure polymers, GLY, MAS, and NIC, (b) Drug loaded (DL)
 361 MAS wafers and (c) Drug loaded (DL) MAS films.

362 The characteristic peaks and band assignments of pure polymers, GLY, MAS, NIC, and

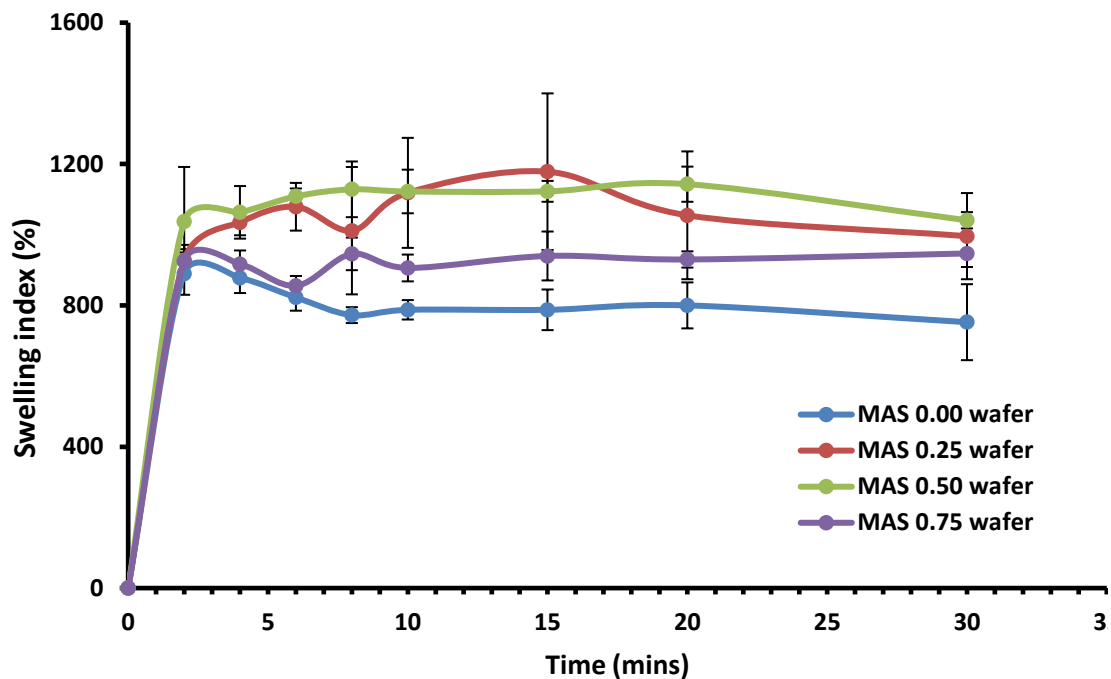
363 NIC loaded composite wafers and films are summarised in Tables A1 and A2
364 respectively (supplementary data). NIC loaded wafers and films demonstrated a shift to
365 higher wavenumber for O-H, O-C=O (asymmetric) and (symmetric) stretching bands.
366 The Si-O-Al (octahedral Al), characteristic peak of MAS at 517cm^{-1} was demonstrated
367 in MAS loaded wafers, with a shift to higher wavelength at 518cm^{-1} , but showed a shift
368 to lower wavenumber at 516cm^{-1} for the corresponding films. However, films without
369 MAS demonstrated a characteristic C-H peak of GLY with a shift to lower wavenumber
370 and C-CH₃ characteristic peak of HPMC (1314cm^{-1}) with a shift to higher wavenumber
371 (1319cm^{-1}).

372 3.7 Swelling

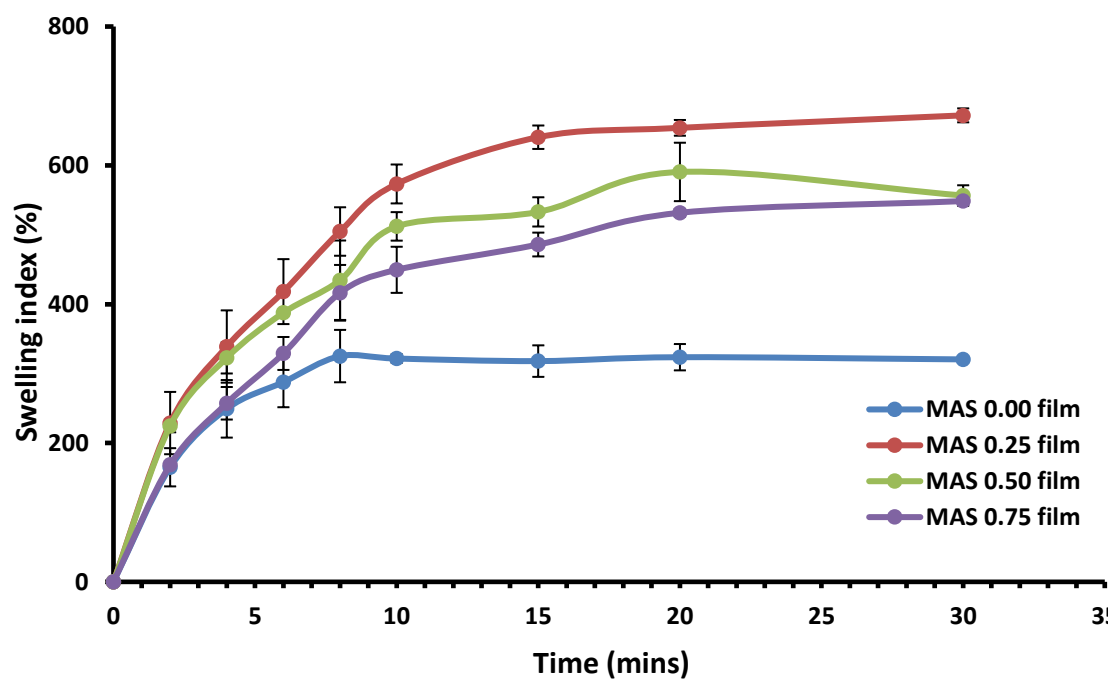
373 Figure 5 shows the swelling profiles of both composite wafers and films
374 containing different concentrations of MAS. Wafers demonstrated a rapid and higher
375 swelling profile (Figure 5a) compared to films (Figure 5b). A swelling index between
376 700 - 1150% was observed in wafers and 150 - 700% in films after 2 mins of contact
377 with PBS solution. Increase in swelling index with incorporation of MAS was
378 demonstrated in both wafers and films. Although MAS wafers (i.e. MAS 0.25, 0.50 and
379 0.75) showed higher swelling index than wafers with no MAS (i.e. MAS 0.00), wafers
380 with MAS 0.75 concentration showed the lowest swelling index among but was still
381 significantly higher ($p=0.0035$) than the wafers with no MAS present. In the same way,
382 films with MAS 0.75 also showed the lowest swelling among the composite films but
383 was still significantly higher ($p=0.0118$) than the films without MAS.

384

385



(a)



(b)

386 **Figure 5** Swelling profiles (i.e. swelling index (%) against time) ($n = 3$) of (a) wafers and (b)
 387 films.

388 3.8 *Mucoadhesion studies*

389 Figure S3 shows the adhesive properties [(PAF, TWA and cohesiveness
390 (stickiness)] of NIC loaded wafers and films. The wafers showed a significant ($p <$
391 0.05) decrease in PAF from $1.29 \pm 0.22\text{N}$ for MAS 0.00 wafer to $0.23 \pm 0.003\text{N}$ for
392 MAS 0.25 wafer, representing about 82% decrease in adhesive force but remained
393 constant with further increase in MAS concentration. NIC loaded films on the other
394 hand, demonstrated an increase in PAF as MAS increased. Films showed an increase
395 from $1.94 \pm 0.13\text{N}$ for MAS 0.00 formulation to $2.44 \pm 0.44\text{N}$ for MAS 0.75. In general,
396 there was a significant difference ($p < 0.05$) in PAF between NIC loaded wafer and
397 film, with the films showing higher PAF compared to their corresponding wafers
398 (Figure S3a). The TWA (Figure S3b) of NIC loaded wafers also demonstrated an initial
399 decrease from $1.01 \pm 0.21\text{Nmm}$ for MAS 0.00 to $0.17 \pm 0.025\text{Nmm}$ for MAS 0.25, and
400 then remained constant as MAS concentration increased which was quite similar to the
401 pattern observed for PAF. NIC loaded films however showed an increase in TWA with
402 in the presence of MAS, increasing from $1.74 \pm 0.52\text{Nmm}$ for MAS 0.25 to 2.28 ± 0.79
403 for MAS 0.75. The cohesiveness (stickiness) profiles of NIC loaded wafers and films
404 are shown in Figure S3c. The cohesiveness of wafers increased with the introduction of
405 MAS, with a value of $1.92 \pm 0.51\text{mm}$ for MAS 0.00 and $9.96 \pm 0.71\text{mm}$ for MAS 0.25.
406 MAS can therefore significantly influence cohesiveness of NIC loaded wafers.
407 However, in NIC film there was no influence, as cohesiveness remained relatively
408 constant as MAS concentration increased.

409 Overall, although NIC loaded composite wafers demonstrated high cohesiveness
410 (stickiness), NIC loaded MAS films demonstrated better mucoadhesive properties
411 considering the PAF and TWA profiles.

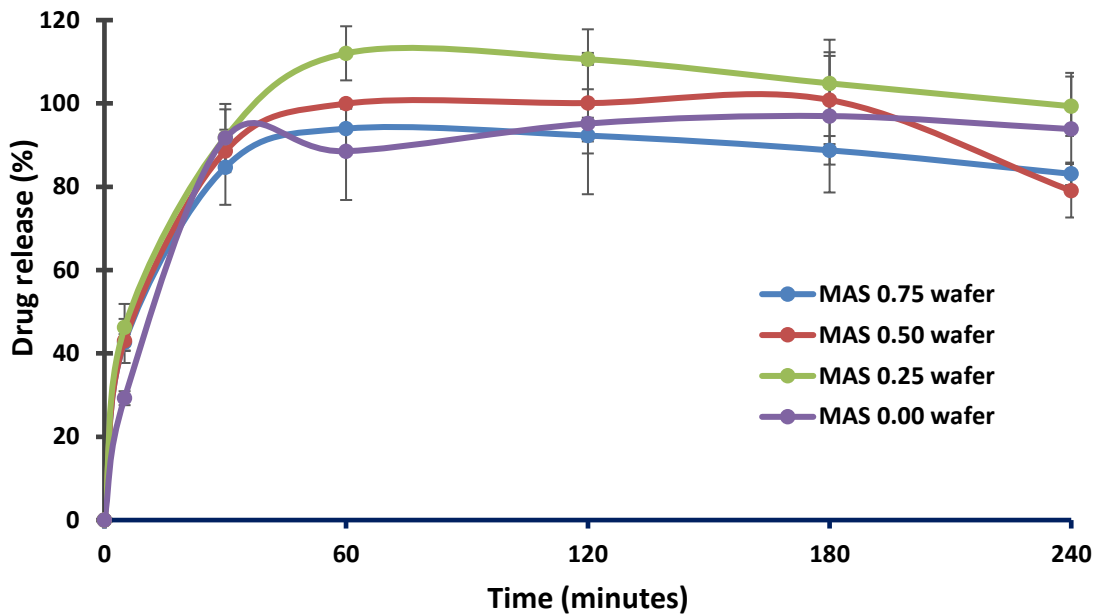
412 **3.9 Drug content (% loading / recovery)**

413 Figure S4 shows the drug content of the composite wafers and films and
414 calculated as percentage drug remaining in the dosage forms after the formulation
415 process. NIC content was $79 \pm 1\%$ and $28 \pm 4\%$ respectively for wafers and films
416 containing no MAS, which increased to 93% and 92% respectively for wafers and films
417 loaded with MAS 0.25, after which both showed a decrease in NIC content as MAS
418 increased further. The increase in MAS from MAS 0.00 to 0.25 had the most significant
419 effect on the NIC content of SA based composite films, with an increase of
420 approximately 70% compared to wafers which increased by 15%. Further, the
421 subsequent decrease in NIC content in composite films as MAS concentration
422 increased, was more pronounced than the corresponding wafers. In the case of wafers,
423 three formulations MAS 0.25 wafers, MAS 0.50 wafers and MAS 0.75 wafers
424 maintained the NIC content above 85% whilst only MAS 0.25 films had values above
425 80%. Due to the very low drug content for MAS films at MAS 0.00, these films were
426 not employed during *in vitro* drug dissolution studies.

427 **3.10 In vitro drug dissolution**

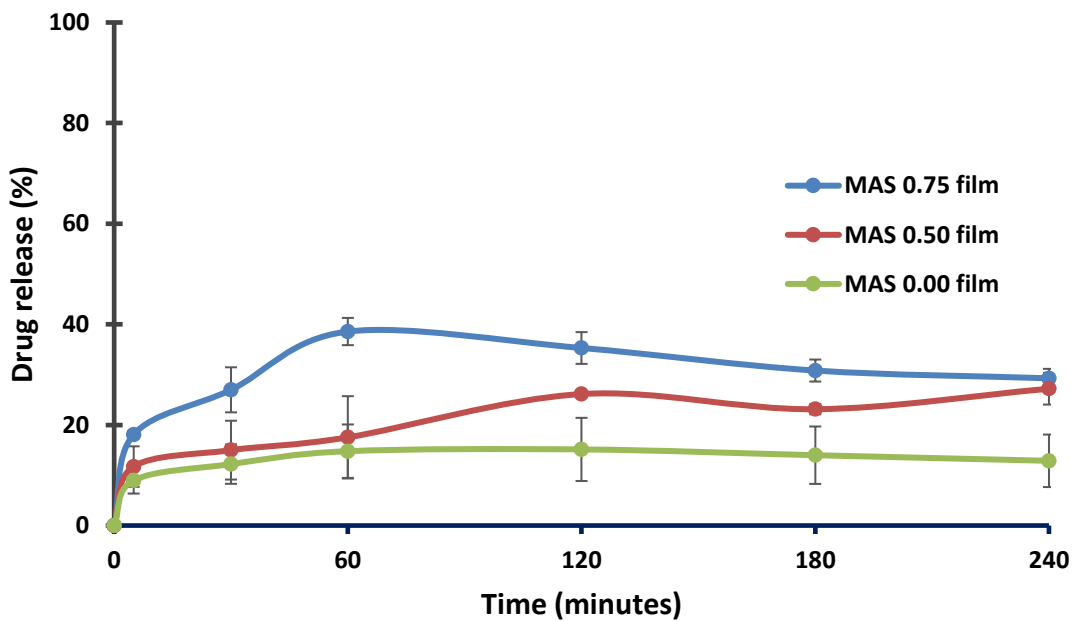
428 Figure 6 shows the drug dissolution profiles of MAS wafers and films. The wafers
429 demonstrated a rapid drug release with about 80-100% of NIC released within 60 mins
430 while films showed a much more sustained release profile with drug gradually released
431 from the polymeric matrix. The different wafer formulations showed similar drug
432 release profiles with no significant difference ($p > 0.05$) observed as MAS
433 concentration increased. However, films demonstrated a significant difference ($p <$
434 0.05) in percentage cumulative drug release as MAS increased. Films containing MAS
435 0.25 showed the slowest release rate with a maximum cumulative drug release of $15.1 \pm$
436 6.3% at 120 mins followed by MAS 0.50 film ($26.1 \pm 0.1\%$) and increased slightly at

437 MAS 0.75 film with a cumulative drug release of $35.6 \pm 2.7\%$.



438

439 (a)



440

441 (b)

442 **Figure 6** *In vitro* drug release profiles ($n = 3$) of NIC loaded (a) wafers and (b) films. containing
443 different MAS concentrations.

444 **3.11 Drug release kinetics**

445 The release parameters of NIC loaded SA based wafers and films have been
446 summarised in Tables A3 and A4 respectively (supplementary data). Based on the R^2
447 values, drug release from wafers fit the Korsmeyer-Peppas best compared to other
448 models. However, the release data for films fit the Korsmeyer-Peppas equation for
449 MAS 0.75 films ($R^2 = 0.8986$) and MAS 0.25 films ($R^2 = 0.9707$) whilst Hixson-
450 Cromwell equation fit the release data for MAS 0.50 films ($R^2 = 0.9947$). The n values
451 of Korsmeyer-Peppas equation in wafers ranged from 0.3306 - 0.4839 and decreased
452 with increase in MAS in wafers and less than 0.45 except for MAS 0.00 wafers
453 (0.4839). Similar to wafers, films demonstrated an n value of less than 0.45, which
454 ranged from 0.1744 - 0.2363.

455

456 **4 Discussion**

457 The introduction of MAS into wafers and film and the presence of SA was to
458 overcome the challenges posed by NIC as regards to volatility and poor stability. The
459 increase in surface stickiness, stringiness and gel strength with increase in MAS
460 concentration was the result of decrease in free volume between the HPMC and SA
461 polymers as the concentration of MAS increased.

462 The mechanical hardness of wafers is related to their handling and friability and
463 therefore consistency of wafer structure can be demonstrated using hardness data as this
464 shows their resistance to compression deformation forces (Boateng & Areago, 2014).
465 The consistency in the hardness for wafers containing MAS 0.00 to 0.50 was attributed
466 to their constant porosities. The decrease in hardness of wafers at higher MAS
467 concentration (MAS 0.75) is due to the increased porosity and low free volume between
468 the polymers due to higher MAS solid particles leading to weaker sponge walls. The

469 internal microstructure (SEM) also demonstrated weak sponge walls in wafers
470 containing the highest MAS concentration (MAS 0.75). It's been reported that an
471 increase in porosity can reduce hardness as a result of reduced interaction between
472 polymer chains within the network (Boateng et al., 2010).

473 The tensile properties of films are very important as they affect ease of handling
474 and application. Pongjanyakul and co-workers demonstrated the effect of MAS on
475 elongation and tensile strength, concluding that addition of solid particles usually
476 decreases films' elongation (Pongjanyakul et al 2005). SA based films showed a
477 decrease in percentage elongation with MAS because MAS reduces the free volume
478 between SA and HPMC (Table 1) which further resulted in the increase in brittleness
479 (tensile strength) and stiffness (elastic modulus). This could imply that MAS had an
480 opposite effect to the known plasticising action of GLY.

481 The physical form of formulations (amorphous or crystalline) can influence
482 functional characteristics such as water uptake and mucoadhesion (Prabaharan & Gong,
483 2008). The crystalline peaks demonstrated in both wafers and films were due to the
484 crystalline nature of the montmorillonite and saponite clay structures of MAS.
485 Although, crystallinity generally decreases dissolution rate, incorporation of MAS
486 increased the swelling index due to the interaction between MAS and SA as
487 demonstrated in ATR-FTIR results and also previously reported (Pongjanyakul et al.,
488 2005). MAS can interact with SA through the formation of hydrogen bonding between
489 surface silanol groups of MAS and the carboxyl groups of SA and the extent of this
490 interaction is responsible for the observed changes in characteristics with increase in
491 MAS concentration.

492 Suitable hydration and swelling play a major role in mucoadhesion as well as
493 drug release patterns (Pawar, Tetteh, & Boateng, 2013). In general, the rapid swelling

494 profile of wafers compared to films was the result of the sponge-like pores in wafers
495 microstructure, enabling faster water ingress and making them hydrate faster than the
496 films. (Pongjanyakul et al., 2005) suggested that the decrease in water uptake in SA
497 films loaded with MAS was due to the interaction of SA and MAS, which produced a
498 denser matrix structure and this could have occurred in the case of the films formulated
499 in this study.

500 SA based films showed higher mucoadhesion than the corresponding wafers due
501 to the presence of GLY. This allowed better contact stage via hydrogen bonding and
502 van der Waals forces (adsorption theory of mucoadhesion) than wafers which were
503 based on the diffusion theory (Smart, 2005). The increase in mucoadhesion in films as
504 MAS concentration increased could be attributed to the exposure of weak positive and
505 negatively charged forces. Upon contact with physiological fluids, the charged MAS
506 interacts with mucin macromolecules leading to increased van der Waals forces and
507 electrostatic interactions (Pongjanyakul & Suksri, 2009, Rowe et al., 2006). The
508 decrease in mucoadhesion of wafers as MAS concentration increased could be due to
509 the poor contact stage caused by gaps related to the sponge-like pores present in wafers
510 (Smart, 2005). In addition, MAS can compete with SA and NIC for binding mucin.
511 However, the increase in MAS showed no noticeable change in adhesion, as the freely
512 available MAS after interaction with NIC, interacts with SA, therefore reducing the
513 availability of the SA cationic group to interact with mucin.

514 The primary aim of incorporating MAS into HPMC-SA wafers and films was to
515 stabilise NIC. The volatility of NIC base is one of the main reasons for its instability in
516 formulations as NIC evaporates at high temperature during the drying process (Nair et
517 al., 1997). MAS can readily interact with amine based drugs through electrostatic
518 interactions which can improve NIC stability (Pongjanyakul & Suksri, 2009). However,

519 higher percentage NIC content was observed in wafers than in the films due to the lower
520 temperatures used during freeze-drying, compared oven drying. The decrease in
521 percentage NIC content in MAS wafers and films at MAS 0.50 and 0.75 can be
522 explained by the increase in repulsive forces which build-up as MAS concentration
523 increased.

524 The release of drug from polymeric matrices such as wafers and films is
525 dependent on factors such as hydration and eventual swelling of the polymeric dosage
526 form (Siepmann & Peppas, 2012). As formulations come in contact with dissolution
527 medium, they undergo hydration, swelling and erosion (dissolution), which was evident
528 in the swelling behaviour of the various wafers and films. The rapid release (80 - 100%
529 in 60 mins) of the wafers corresponded to the high swelling index, due to the sponge-
530 like porous internal structure of wafers (SEM and percentage porosity). Therefore, the
531 use of SA based wafers can be efficient in achieving rapid release of NIC to the buccal
532 mucosa to ensure rapid easing of the urge to smoke tobacco. The much slower release
533 of NIC from the films, which corresponded to low swelling index, can be important in
534 achieving sustained release of NIC, with an extended effect to reduce the need for
535 frequent administration. The release exponents of MAS loaded formulations of less than
536 0.45 was outside the limits of Korsmeyer-Peppas model and also highlights the
537 limitations of the Korsmeyer-Peppas model in the understanding of drug release
538 mechanisms (Shoaib, Tazeen, Merchant, & Yousuf, 2006). However, the release
539 exponent of 0.48 for wafers without MAS (MAS 0.00 wafers) shows that drug release
540 from these wafers followed a Fickian diffusion transport mechanism (Nair et al., 2013).

541 **5 Conclusions**

542 Composite SA based films and wafers, incorporating MAS have been successfully

543 formulated as potential buccal delivery systems for NRT. The two formulations
544 demonstrated different behaviours in their functional physical characteristics. The
545 wafers showed a porous internal morphology which contribute to higher swelling index
546 than continuous sheet of films. MAS improved the physical stability of NIC with an
547 increase in drug loading capacity via molecular interaction between the inorganic clay
548 and the alkaline drug. The release of drug from the wafers was rapid while release from
549 the corresponding films was sustained. The MAS stabilized formulations have great
550 potential as buccal delivery systems for NRT.

551

552 **References**

- 553 Adhikari, S.N.R., Nayak, B.S., Nayak, A.K. & Mohanty, B. (2010). Formulation and
554 evaluation of buccal patches for delivery of atenolol. *AAPS PharmSciTech*, *11*, 1038-
555 1044.
- 556
557 Adrian, C.L., Olin, H.B.D., Dalhoff, K. & Jacobsen, J. (2006). In vivo human buccal
558 permeability of nicotine. *International Journal of Pharmaceutics*, *311*, 196-202.
- 559
560 Aguzzi, C., Cerezo, P., Viseras, C. & Caramella, C. (2007). Use of clays as drug
561 delivery systems: Possibilities and limitations. *Applied Clay Science*, *36*, 22-36.
- 562
563 Benowitz, N.L., Jacob, P. & Savanapridi, C. (1987). Determinants of nicotine intake
564 while chewing nicotine polacrilex gum. *Clinical Pharmacology & Therapeutics*, *41*,
565 467-473.
- 566
567 Boateng, J.S. & Areago, D. (2014). Composite Sodium Alginate and Chitosan Based
568 Wafers for Buccal Delivery of Macromolecules. *Austin Journal of Analytical and*
569 *Pharmaceutical Chemistry*, *1*, 1022.
- 570
571 Boateng, J.S., Auffret, A.D., Matthews, K.H., Humphrey, M.J., Stevens, H.N.E. &
572 Eccleston, G.M. (2010). Characterisation of freeze-dried wafers and solvent evaporated
573 films as potential drug delivery systems to mucosal surfaces. *International Journal of*
574 *Pharmaceutics*, *389*, 24-31.
- 575
576 Boateng, J.S. & Okeke, O. (2014). Chitosan-based films for the sustained release of
577 peptides: a new era in buccal delivery? *Therapeutic delivery*, *5*, 497-500.
- 578
579 Dash, S., Murthy, P.N., Nath, L. & Chowdhury, P. (2010). Kinetic modeling on drug
580 release from controlled drug delivery systems. *Acta Pol Pharm*, *67*, 217-223.
- 581

582 Gilman, J.W. (1999). Flammability and thermal stability studies of polymer layered-
583 silicate (clay) nanocomposites1. *Applied Clay Science*, 15, 31-49.

584

585 Khan, S., Boateng, J.S., Mitchell, J. & Trivedi, V. (2015). Formulation,
586 Characterisation and Stabilisation of Buccal Films for Paediatric Drug Delivery of
587 Omeprazole. *AAPS PharmSciTech*, 1-11.

588 Manivannan, R., Balasubramaniam, A., Anand, D.C., Sandeep, G. & Rajkumar, N.
589 (2008). Formulation and in-vitro evaluation of mucoadhesive buccal tablets of diltiazem
590 hydrochloride. *Research Journal of Pharmacy and Technology*, 1, 478-480.

591

592 Mihranyan, A., Andersson, S.-B. & Ek, R. (2004). Sorption of nicotine to cellulose
593 powders. *European Journal of Pharmaceutical Sciences*, 22, 279-286.

594

595 Morales, J.O. & McConville, J.T. (2011). Manufacture and characterization of
596 mucoadhesive buccal films. *European Journal of Pharmaceutics and*
597 *Biopharmaceutics*, 77, 187-199.

598

599 Nair, A.B., Kumria, R., Harsha, S., Attimarad, M., Al-Dhubiab, B.E. & Alhaider, I.A.
600 (2013). In vitro techniques to evaluate buccal films. *Journal of Controlled Release*, 166,
601 10-21.

602

603 Nair, M.K., Chetty, D.J., Ho, H. & Chien, Y.W. (1997). Biomembrane permeation of
604 nicotine: Mechanistic studies with porcine mucosae and skin. *Journal of*
605 *Pharmaceutical Sciences*, 86, 257-262.

606

607 Okeke O.C., Boateng, J.S. (2016). Composite HPMC and sodium alginate based buccal
608 formulations for nicotine replacement therapy. *International Journal of Biological*
609 *Macromolecules*. 91, 31-44.

610

611 Pandey, S.L., Hingawe, N.T., Das, U. & Patil, A.T. (2014). Mucoadhesive buccal
612 tablets of domperidone: formulation evaluation and effects of process variables. *Journal*
613 *of Pharmaceutical Investigation*, 44, 103-110.

614

615 Pavlidou, S. & Papaspyrides, C.D. (2008). A review on polymer-layered silicate
616 nanocomposites. *Progress in Polymer Science (Oxford)*, 33, 1119-1198.

617

618 Pawar, H.V., Tetteh, J. & Boateng, J.S. (2013). Preparation, optimisation and
619 characterisation of novel wound healing film dressings loaded with streptomycin and
620 diclofenac. *Colloids and Surfaces B: Biointerfaces*, 102, 102-110.

621

622 Pongjanyakul, T., Pripem, A. & Puttipipatkachorn, S. (2005). Investigation of novel
623 alginate–magnesium aluminum silicate microcomposite films for modified-release
624 tablets. *Journal of Controlled Release*, 107, 343-356.

625

626 Pongjanyakul, T. & Suksri, H. (2009). Alginate-magnesium aluminum silicate films for
627 buccal delivery of nicotine. *Colloids and Surfaces B: Biointerfaces*, 74, 103-113.

628

629 Pongjanyakul, T. & Suksri, H. (2010). Nicotine-loaded sodium alginate–magnesium
630 aluminum silicate (SA–MAS) films: Importance of SA–MAS ratio. *Carbohydrate*
631 *Polymers*, 80, 1018-1027.

632
633 Prabakaran, M. & Gong, S. (2008). Novel thiolated carboxymethyl chitosan-g- β -
634 cyclodextrin as mucoadhesive hydrophobic drug delivery carriers. *Carbohydrate*
635 *Polymers*, 73, 117-125.
636
637 Rakić, V., Damjanović, L., Rac, V., Stošić, D., Dondur, V. & Auroux, A. (2010). The
638 adsorption of nicotine from aqueous solutions on different zeolite structures. *Water*
639 *Research*, 44, 2047-2057.
640
641 Rowe, R.C., Sheskey, P.J., Owen, S.C. & American Pharmacists, A. (2006). *Handbook*
642 *of Pharmaceutical Excipients*. Pharmaceutical Press.
643
644 Sattar, M., Sayed, O.M. & Lane, M.E. (2014). Oral transmucosal drug delivery –
645 Current status and future prospects. *International Journal of Pharmaceutics*, 471, 498-
646 506.
647
648 Shoaib, M.H., Tazeen, J., Merchant, H.A. & Yousuf, R.I. (2006). Evaluation of drug
649 release kinetics from ibuprofen matrix tablets using HPMC. *Pakistan Journal of*
650 *Pharmaceutical Sciences*, 19, 119-124.
651
652 Siepmann, J. & Peppas, N.A. (2012). Modeling of drug release from delivery systems
653 based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews*,
654 64, Supplement, 163-174.
655 Singhvi, G. & Singh, M. (2011). Review: in-vitro drug release characterization models.
656 *International Journal of Pharmacy Studies Research*, 2, 77-84.
657
658 Smart, J.D. (2005). The basics and underlying mechanisms of mucoadhesion. *Advanced*
659 *Drug Delivery Reviews*, 57, 1556-1568.
660
661 İkinçi, G., Şenel, S., Wilson, C.G. & Şumnu, M. (2004). Development of a buccal
662 bioadhesive nicotine tablet formulation for smoking cessation. *International Journal of*
663 *Pharmaceutics*, 277, 173-178.
664