



## Effect of pH, Time and Temperature on Forced Degradation Studies of Quercetin in Presence of Polymers

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This study investigated the influence of pH, time and temperature on the degradation and adsorption of quercetin by molecularly imprinted polymer beads and non-imprinted polymers. Molecularly imprinted polymers and non-imprinted polymers were prepared by precipitation polymerization using acrylamide and 4-vinylpyridine co-monomers and ethylene glycol dimethacrylate (EGDMA) cross-linker. The polymers were characterized by Fourier transform infrared spectroscopy, thermogravimetric analysis, scanning electron microscopy and elemental analysis. Adsorption/degradation studies were carried out at varying pH, time and temperature. Maximum quercetin adsorption by molecularly imprinted polymer and non-imprinted polymer was observed at pH 4 (20 °C) and pH 6 (60 °C). Highest degradation in blank solutions was seen at pH 2 at 60 °C after 120 h. HPLC analysis showed the presence of degradation products. Quercetin was released back into solution in some instances which arose a potential of the prepared molecularly imprinted polymers in being used as future prospect in drug delivery systems.

**Keywords:** Quercetin, Adsorption, Degradation, Imprinted polymer.

### INTRODUCTION

Quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one] is a bioactive flavonoid compound found in many common food stuffs such as nuts, vegetables and fruits. It is also known for its diverse pharmacological effects including antioxidant, anticancer, anti-inflammatory activities and protection against cardiovascular diseases [1]. However, quercetin in plant materials is usually present as glycosides and it requires to be degraded into its metabolites for bio-absorption [2]. Some times, the process of cooking food may result in degradation of flavonoids into their corresponding metabolites [3]. To test the stability of the drug or its metabolites, usually forced degradation studies also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. are carried out under severe conditions [4]. Charde *et al.* [4] defined forced degradation as a process of degrading drug products under more severe conditions and the study to determine the stability of produced degradation products. Several degradation conditions including photolytic, thermal, thermal/humidity, acid-base hydrolysis and oxidative stress could be used to test the stability of drug substances [5]. Zvezdanovic *et al.* [6] observed that quercetin was more susceptible to UV-irradiation induced degradation compared to rutin. The stability

of rutin was attributed to the hydroxyl group on the C-ring of quercetin. Degradation of quercetin aglycones is known but unfortunately it is less reported [7] probably because the mechanism of such degradation is still very much debatable [6]. Degradation of quercetin in aqueous solution at pH  $\geq$  5 has been reported with 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxybenzoic acid being the major degradation products [8]. It was demonstrated that complexation of quercetin with  $\beta$ -cyclodextrin derivatives greatly improved the solubility and stability of quercetin under alkaline pH conditions [9]. In the present studies, acid-base hydrolysis coupled with thermal treatment was used to study the degradation of quercetin in the presence of imprinted polymer particles.

Polymer hydrophobicity (and aqueous solubility) could fast-track degradation but the mechanism by which polymers impact degradation is complex. Because molecularly imprinted polymers (MIPs) are hydrophobic in nature, therefore, the current study was aimed at assessing whether MIPs would play a role in quercetin degradation during adsorption of quercetin onto MIPs. Molecularly imprinted polymers are polymers prepared in the presence of a template molecule (that is removed once the polymer has formed) to mimic the enzyme substrate-receptor principle. Once the template has been removed it creates cavities that are complementary to the template

molecule in terms of shape, size and functionalities [10]. Only the template can rebind specifically to the generated adsorption sites. Molecularly imprinted polymers for the recognition of quercetin from various samples have been reported [10-13]. In addition to MIPs being used as chromatographic sorbents [10], sensors [14,15], catalysis [15], environmental analysis [16], *etc.* molecularly imprinted polymers are also being explored as drug delivery assays [17]. The important fact about drug delivery assays is that, MIPs used should adsorb the target molecule and release it back under certain controlled conditions. The MIPs used in this study were prepared by precipitation polymerization. The present study seeks to address whether the effect of temperature at different pH and prolonged time will influence the adsorption of quercetin on MIPs or will it cause release back of quercetin followed by degradation.

### EXPERIMENTAL

Quercetin, 4-vinylpyridine, ethylene glycol dimethacrylate (EGDMA), 1,1'-azobis(cyclohexane carbonitrile) (ACCN) initiator, acetic acid, methanol and tetrahydrofuran (THF) were supplied by Sigma-Aldrich (Johannesburg, South Africa). Hydrochloric acid and sodium hydroxide were purchased from Merck Chemical Co (Johannesburg, South Africa). All solutions were made from the ultrapure water from LaboStar equipment by Siemens (Warrendale, Pennsylvania, USA). ADWA AD111 OPR pH meter from Adwa Instruments (Szeged, Hungary) was used to measure the pH of the solutions. All experiments

were carried out in duplicate using an MS-53M Multichannel stirrer from Jeio Tech (Seoul, Korea).

**Preparation of molecular imprinted polymer and non-imprinted polymer:** Molecular imprinted polymers were prepared using precipitation polymerization according to the following synthesis protocol: quercetin (0.4 mmol), acrylamide (1 mmol) and 4-vinylpyridine (4 mmol) were dissolved in 80 mL of THF/H<sub>2</sub>O/methanol (6:3:1, v/v/v) mixture and stirred for 30 min at room temperature to form a template-complex. The reaction mixture was then put on ice, ethylene glycol dimethacrylate (9 mmol) and 100 mg 1,1'-azobis(cyclohexane carbonitrile) were added to the reaction mixture while it was being stirred on ice. The reaction mixture was purged with N<sub>2</sub> for 10 min to remove oxygen and to prevent polymerization at low temperature. The reaction vessel was then removed from ice and polymerization was initiated at 80 °C in an oil bath for 12 h. After polymerization, particles were sedimented in acetone to remove unreacted monomers and fine particles. The quercetin template was then removed using a heated reflux extraction in 100 mL of methanol-acetic acid mixture 9:1 (v/v) as an extraction solvent in a Soxhlet extraction set-up. Thereafter, particles were dried in an oven at 105 °C for 12 h and kept for use. Non-imprinted polymers were prepared exactly the same way but the template was omitted. The schematic diagram for the preparation of MIP is depicted in Fig. 1.

A Perkin Elmer spectrum 400 FT-IR/FT-NIR spectrometer (Waltham, MA, USA) was used to record the absorption spectra

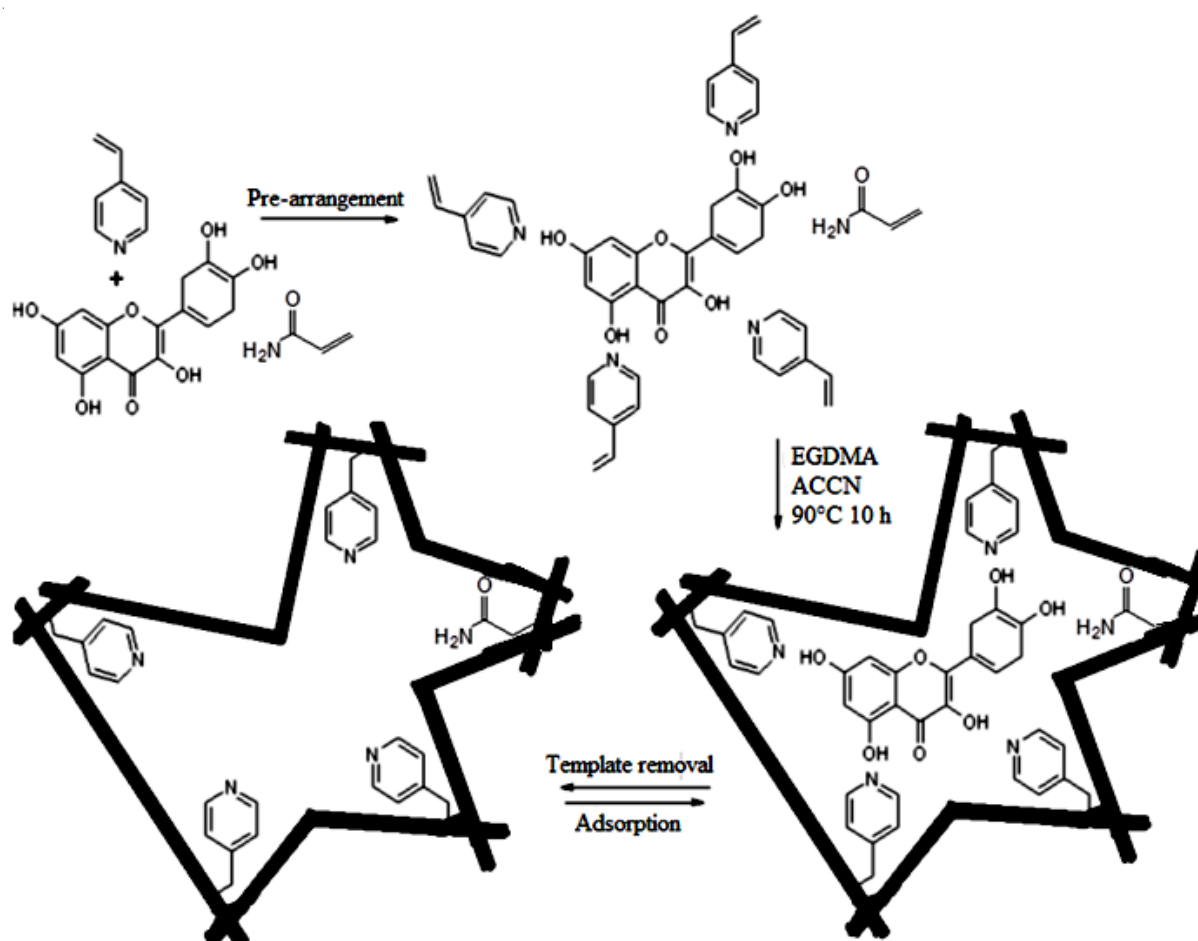


Fig. 1. Quercetin MIP preparation

of imprinted polymers. FEI Nova NanoLab FIB/SEM (Milpitas, CA, USA) was used to gain information on the morphology and surface texture of polymers. Elemental analysis of imprinted polymers was collected on a Thermo Flash 2000 series CHNS/O Organic Elemental Analyser.

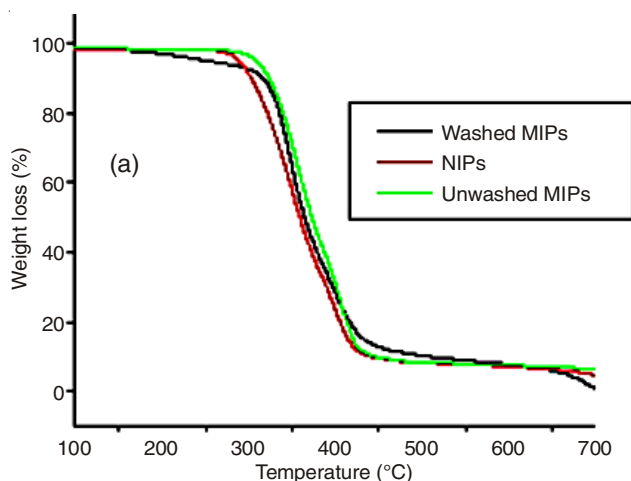
#### Batch rebinding-adsorption-degradation studies:

Quercetin solutions (100 mg/L) of different pHs (pH 2-9) were prepared in 250 mL volumetric flasks from a stock solution using a methanol/water mixture. The pH was adjusted using diluted solutions of HCl or NaOH and recorded at 25 °C. About 0.05 g of MIPs was weighed and transferred into a 100 mL beaker and 30 mL of 100 mg/L solution was added to MIPs and stirred for 120 h. Sample aliquots of 1 mL were taken from each beaker at different sampling times (*i.e.*, 24, 72 and 120 h). The sample aliquots were then filtered using solid phase extraction vacuum filtration then analyzed with high performance liquid chromatography with diode array detector (HPLC-DAD) (Agilent Technologies, 1260 Infinity Series). The same procedure was performed for the NIPs. As a control, quercetin solution prepared as above was stirred for 24, 72 and 120 h, and analyzed on the HPLC at 280 and 370 nm wavelength. In these control solutions no MIPs or NIPs were added. The percentage adsorption of quercetin adsorbed by MIP and NIP was calculated from eqn. 1:

$$R (\%) = \left( \frac{(C_o - C_e) \text{ in polymer}}{C_o} - \frac{(C_o - C_e) \text{ in blank}}{C_o} \right) \times 100$$

where  $C_o$  is the initial concentration (mg/L) and  $C_e$  is the final concentration (mg/L) of quercetin.

**Quantitative determination of quercetin:** Quantitative determination of quercetin in solution after adsorption/degradation was performed on an HPLC-DAD consisting an Agilent Zorbax SB-C18 column (100 mm × 2.1 mm, 3.5 μm), a methanol/water (50:50, v/v) mobile phase containing formic acid (10 %) and a flow rate of 1 mL/min was used. The injection volume was 20 μL and detection was accomplished at 280 and 370 nm, respectively. The quantification of quercetin was performed using a five-point calibration curve of standards at concentrations between 1 and 20 mg/L. Each vial taken to analysis had a total volume of 1 mL.



## RESULTS AND DISCUSSION

**FTIR characterization:** Fig. 2 shows the FTIR spectra of washed MIP, unwashed MIP and NIP. It can be observed that all spectra show similar backbone due to the high concentration of ethylene glycol dimethacrylate used in the synthesis [11,12]. In all FTIR spectra, strong vibration bands at 1720 and 1110  $\text{cm}^{-1}$  were attributed to the C=O and C-O functional groups of ethylene glycol dimethacrylate crosslinker. The diminished nature of vibrational band at 1637  $\text{cm}^{-1}$  attributed to C=C bonds of monomers implied that there were fewer unpolymerized C=C present in the polymers [18]. The vibrational stretch bands at 2982  $\text{cm}^{-1}$  were attributed to the C-H bands of the functional monomers and crosslinker.

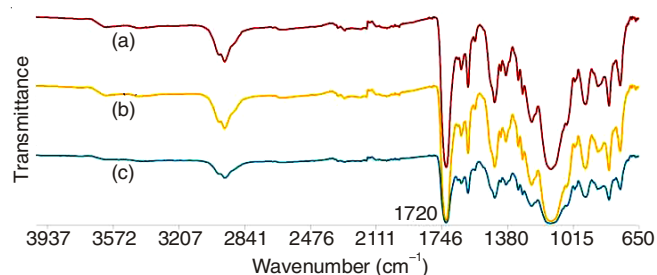


Fig. 2. FTIR spectra of the (a) NIPs, (b) washed MIPs and (c) unwashed MIPs

**Thermogravimetric analysis:** Thermogravimetric analysis was used to study the different decomposition stages of the polymer particles. Fig. 3 shows the different thermograms of the MIPs (washed and unwashed) and the corresponding NIP. Different degradation patterns were observed and it was noted that all polymer particles started to degrade at about 300 °C. This decomposition amounted to about 95 % weight loss in all polymers and attributed to the decomposition of monomers and crosslinker. The exact decomposition temperatures were 330 °C for the washed MIP and NIPs and 370 °C for the unwashed MIP. The washed MIP and the NIP had lower temperatures of decomposition indicating lower thermal stability (Fig. 3b). This could be due to lower molecular weight compared to the unwashed MIP that still had quercetin in them.

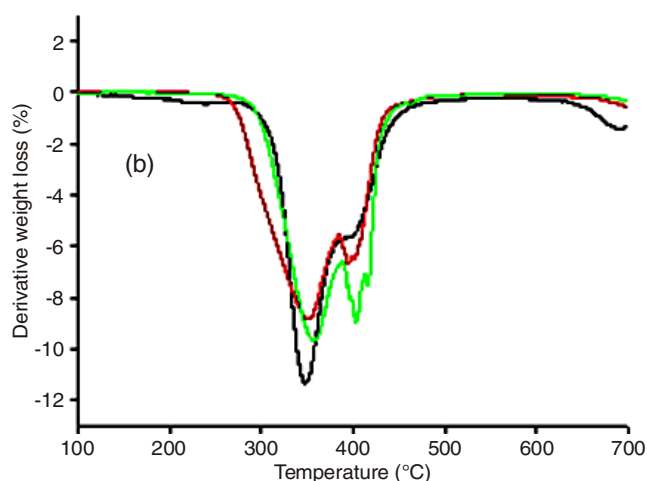


Fig. 3. TGA curves of washed, unwashed MIPs and NIPs. (a) The weight loss as a function of temperature and (b) the derivative weight loss as a function of temperature

**Scanning electron microscope analysis:** Fig. 4 shows SEM micrographs for MIP and NIP. It can be observed that the MIP particles produced by the precipitation method were monodispersed bar some few big lumps which were separated by hand before experiments (Fig. 4a). On the other hand, NIP particles were agglomerated into big lumps but one can see that those lumps were made of tiny beads (Fig. 4b). Therefore, prior adsorption experiments were conducted NIP particles were crushed mildly with mortar and pestle to obtain poly-dispersed beads. Solvent evaporation during polymer synthesis and the speed of stirring may lead to agglomeration.

**Elemental analysis:** Elemental analysis of washed MIP, unwashed MIP and NIP was carried out to observe the changes in the polymer backbone as a result of imprinting. Table-1 showed that the percentages of CHN elements for all samples were similar. Further enhancing what was observed with FTIR that polymers had similar backbone. The high %N (2.05 %) in unwashed MIP was attributed to presence of unreacted 4-vinylpyridine and/or acrylamide monomers. Also, the unwashed MIP showed a slightly higher percentage of carbon (64.81 %) and hydrogen (7.71 %) attributable to the presence of quercetin template which was extracted in the washed MIP and not added in NIP. The closeness of %CHN values for washed MIP and NIP signified that the quercetin template was removed efficiently.

TABLE-1  
ELEMENTAL ANALYSIS OF UNWASHED  
MIP, WASHED MIP AND NIP

Polymer	Elemental analysis (%)		
	C	H	N
Washed MIP	63.76	7.44	1.69
Unwashed MIP	64.81	7.71	2.05
NIP	63.34	7.49	1.66

### Adsorption and degradation studies

**Effect of solution pH and temperature:** The influence of pH on the adsorption of quercetin by MIP and NIP was

investigated at 20, 40 and 60 °C, and the results of these are depicted in Table-2, respectively. The initial concentration of the quercetin solution was 100 mg/L and all experiments were performed in duplicate. After the reaction time had elapsed, samples were withdrawn and analyzed for quercetin using an HPLC method. A typical calibration curve for quercetin standards obtained from the HPLC is shown in Fig. 5. A correlation coefficient ( $R^2$ ) of 0.999 was achieved and this showed good precision and linearity.

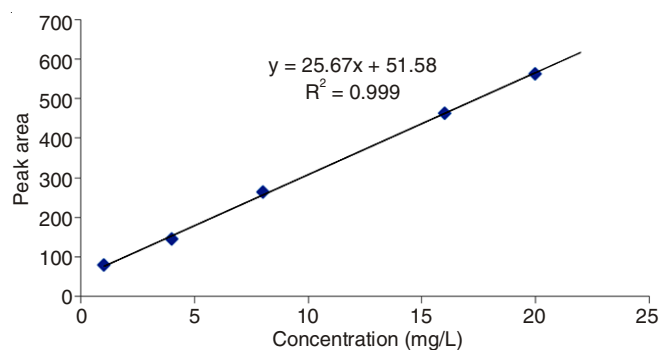


Fig. 5. HPLC calibration curve for quercetin standards

### Adsorption/degradation studies carried out at 20 °C:

The adsorption of quercetin by MIP and NIP as well as the effect of degradation was investigated at pH 2, 4, 6 and 9 for 5 days while stirring at 20 °C. The results are displayed in Table-2. Equilibrium quercetin concentrations at pH 2 and 4 for MIP and NIP samples increased with increasing sampling times. The gradual increase of quercetin concentration in the solutions containing MIP and NIP implied that quercetin molecules were first adsorbed then released back into solution. The amount released back into solution by MIP was 2 % lower than that released by NIP after 5 days. The lower amount of quercetin released by MIP indicated that MIP had a better quercetin retention at pH 2 and 4 due to imprinting effects. The per cent quercetin adsorbed by MIP at pH 2 decreased from 65.0 % in 24 h to 45.4 % in 120 h. On the other hand, the percentage of

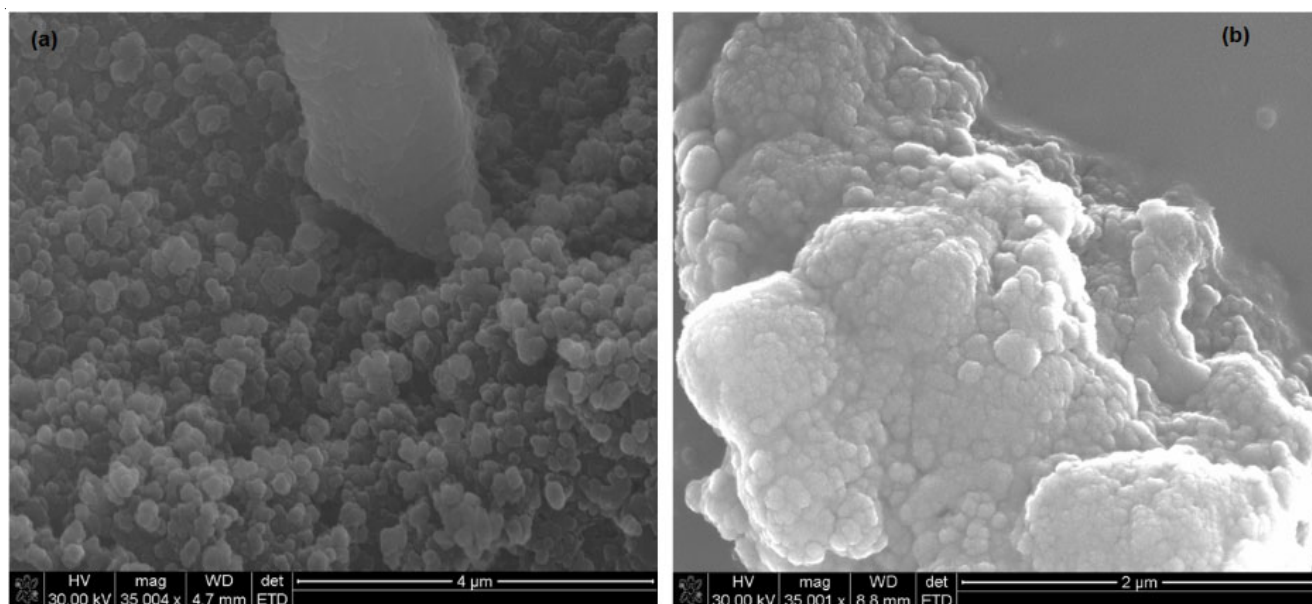


Fig. 4. SEM micrograph for MIP(a) and NIP (b)



TABLE-2  
CONCENTRATION OF QUERCETIN (mg/L) LEFT IN SOLUTION AT DIFFERENT SAMPLING TIMES AND pH VALUES AT DIFFERENT TEMPERATURE. INITIAL QUERCETIN CONCENTRATION WAS 100 mg/L. IN BRACKETS ARE THE % ADSORPTION OF QUERCETIN BY MIP AND NIP OR % DEGRADATION IN THE CASE OF BLANK SOLUTION

	Temperature: 20 °C				Temperature: 40 °C				Temperature: 60 °C			
	pH 2	pH 4	pH 6	pH 9	pH 2	pH 4	pH 6	pH 9	pH 2	pH 4	pH 6	pH 9
Day 1 (24 h)												
MIP (mg/L)	5.79 (65.03)	10.80 (64.57)	37.10 (31.36)	95.16 (-20.42)	34.06 (22.50)	43.55 (46.36)	73.02 (25.23)	92.35 (1.86)	13.95 (53.39)	25.42 (46.59)	48.24 (10.83&)	57.64 (35.63)
NIP (mg/L)	33.64 (37.18)	13.83 (61.62)	50.01 (18.45)	36.50 (38.24)	52.26 (4.30)	45.26 (44.65)	81.60 (16.65)	96.52 (-2.31)	21.37 (45.97)	46.21 (25.80)	52.73 (6.34)	66.49 (26.80)
Blank (mg/L)	70.82 (29.18)*	75.45 (24.55)*	68.46 (31.54)*	74.74 (25.26)*	56.56 (43.44)*	89.91 (10.09)*	98.25 (1.75)*	94.21 (5.79)*	67.34 (32.66)*	72.01 (27.99)*	59.07 (40.93)*	93.29 (6.97)*
Day 3 (72 h)												
MIP (mg/L)	6.24 (59.82)	22.46 (52.92)	26.01 (38.90)	89.33 (-18.92)	29.82 (8.79)	32.05 (53.23)	70.52 (25.61)	82.23 (9.33)	43.24 (-9.25)	64.13 (-10.29)	55.29 (-17.93)	77.82 (-18.34)
NIP (mg/L)	39.99 (26.07)	28.87 (46.51)	32.49 (32.42)	34.27 (36.14)	49.96 (-11.35)	40.45 (44.83)	45.01 (51.12)	75.68 (15.88)	72.46 (-38.47)	42.93 (10.91)	74.11 (-36.75)	45.33 (13.76)
Blank (mg/L)	66.06 (33.94)*	75.38 (24.62)*	64.91 (35.09)*	70.41 (29.59)*	38.61 (61.39)*	85.28 (14.72)*	96.13 (3.87)*	91.56 (8.44)*	33.99 (66.01)*	53.84 (46.16)*	37.36 (62.75)*	59.09 (40.91)*
Day 5 (120 h)												
MIP (mg/L)	14.25 (45.37)	29.40 (46.06)	21.55 (39.35)	83.95 (-15.21)	23.23 (-0.82)	24.90 (55.75)	33.58 (51.65)	74.01 (15.47)	18.73 (1.33)	32.74 (3.79)	37.81 (-3.56)	25.97 (18.34)
NIP (mg/L)	41.25 (18.37)	29.77 (45.69)	28.67 (32.23)	32.61 (36.13)	31.36 (-8.95)	50.78 (29.87)	35.85 (49.38)	86.25 (3.23)	25.15 (-5.09)	21.76 (14.77)	20.46 (13.79)	54.08 (-9.07)
Blank (mg/L)	59.62 (40.38)*	75.46 (24.54)*	60.90 (39.10)*	68.74 (31.26)*	22.41 (77.59)*	80.65 (19.35)*	85.23 (14.77)*	89.48 (10.52)*	20.06 (79.94)*	36.53 (63.47)*	34.25 (65.75)*	44.31 (55.69)*

\*represent % degraded

quercetin adsorbed by NIP at pH 2 decreased from 37.2 % in 24 h to 18.4 % in 120 h. The difference was attributed to the adsorption of quercetin by MIP. However, at pH 4 the per cent quercetin adsorbed by MIP and NIP was similar. It has been shown by UV-visible spectrophotometer studies that the absorption maxima and colour of solution changed when the pH of solutions were adjusted by hydrochloric acid and sodium hydroxide [19]. These changes were attributed to formation of decomposition products [19] of quercetin in water/ethanol solution.

In blank solutions, the concentration of quercetin in solution decreased slightly with sampling times at pH 2, while a more stable concentration of quercetin was observed at pH 4. The percentage degradation of quercetin at pH 4 in blank solutions was 24.55 % at 24 h, 24.62 % at 72 h and 24.54 % at 120 h. These results indicated that part of quercetin was degraded after stirring quercetin solution at 20 °C (pH 4) for 120 h while other part was intact.

At pH 6 and 9, quercetin concentration left in solution decreased with increasing sampling times, both in the presence and absence of polymer particles. The concentration of quercetin left in solution after day 1 at pH 6 and 9 was much higher than that left at pH 2 and 4 for MIPs. For NIP, the amount left at pH 2 was comparable to pH 9, while for the blank solution the concentration of quercetin left after day 1 were all similar from pH 2 to 9. This showed that the pH did not play a significant role in degrading quercetin at 20 °C in the first 24 h. No significant change in the per cent quercetin adsorbed by MIP at pH 6 was observed in day 3 (38.90 %) and day 5 (39.35 %). The same trend was observed for NIP samples at pH 6. It seems that the presence of MIP inhibited the degradation of quercetin at pH 9 as the rate of degradation in blank solution was higher than adsorption rate, hence the negative sign observed. Adsorption of quercetin onto MIP was more favoured at pH 6 than pH 9.

The lower adsorption of quercetin by MIP and NIP at pH 9 can be attributed to the deprotonation of quercetin in alkaline conditions. The deprotonation of quercetin will cause the bonding interactions between quercetin and 4-vinylpyridine or acrylamide functional groups to be weakened. Furthermore, about 40 and 30 % was attributed to degradation at pH 6 and 9 for blank solutions, respectively. At pH 9, the presence of NIP facilitated more adsorption or degradation as the amount of quercetin remaining in solution was relatively lower than in MIP and blank solution after 5 days. The negative values in brackets in Table-2 indicate that the rate of degradation in the blank solution was higher than the rate of adsorption/degradation in the presence of MIP or NIP particles. Per cent adsorption by MIP decreased as the pH increased from pH 2-9. Therefore, it can be said that solution pH plays a vital role in the adsorption and degradation of quercetin. Also, Chebotarev and Snigur [20] demonstrated that quercetin exists in different ionic forms at different pH values. Quercetin was likely to exist as neutral molecule at pH 1.8-4.0 then losses each hydroxyl proton as the pH was increased from 5 to 11 [20].

**Adsorption carried out at 40 °C:** When the reaction solutions were stirred at 40 °C, a similar pattern was observed in both acidic and basic conditions, with and without polymer particles. That is, the concentration of quercetin left in solutions decreased as the sampling times increased from 24 h to 120 h for all pHs. Highest decrease of quercetin in solution was observed at pH 2 for all the samples after 5 days. About 78 % (blank solution) had degraded at pH 2 after 5 days. Taking into consideration this amount degraded at pH 2, one can infer that initially quercetin was adsorbed on MIP and NIP (amount left in blank after day 1 was higher than the amount left in MIP and NIP solutions) then released back into solution as the time progressed and then subsequently degraded.

However, at pH 4 the amount of quercetin attributed to degradation was about 19 % in blank solution after 5 days. This meant that a significant portion of quercetin was adsorbed by MIP (56 %) whereas only 29 % was adsorbed by NIP after day 5 at pH 4. The per cent of quercetin adsorbed by MIP after day 5 was about 2-folds greater than that in NIP. Therefore, the lower amounts of quercetin left in solution in the presence of MIP and NIP could be attributed to adsorption rather than degradation. At pH 6 only about 15 % of quercetin degraded after 5 days but 52 and 49 % was adsorbed MIP and NIP, respectively. Very low amounts of quercetin adsorption were achieved at pH 9 by both MIP and NIP after 5 days. The degradation in the blank solutions was also much lower than that observed at pH 9 for reactions carried out at 20 °C.

**Adsorption carried out at 60 °C:** Table-2 displays the results obtained after conducting adsorption/degradation studies at 60 °C for 5 days. The percentage of quercetin degradation in blank solutions increased as the sampling time increased for all pH values studied and the highest percentage degradation (80 %) was observed at pH 2. This was a similar trend to the results obtained at 20° and 40 °C for blank solutions. The per cent of quercetin degraded at 60 °C was higher than the amounts observed at 20 and 40 °C in all the studied pHs after 5 days, signifying that temperature played a role in quercetin degradation. These results were in agreement with Buchner *et al.* [21] and Dall'Acqua *et al.* [22] who noted that the stability of quercetin in water and ethanol medium was limited at higher temperatures. At pH 2 and 4 (MIP and NIP solutions), the quercetin concentrations decreased after day 1, then a sudden increase in the amount of quercetin in solution was observed at day 3 and a subsequent decrease in day 5. The sudden increase of quercetin concentration signified a release back of quercetin molecules into solution. The higher per cent degradation (80 %) observed in blank solution at pH 2 suggest that quercetin released back from MIP and NIP undergone degradation, hence lower amounts at pH 2 after day 5. The adsorption and release back of quercetin is a property that can be explored for drug delivery systems by imprinted polymers.

At pH 6 and 9, in some instances the rate of degradation was higher than the rate of adsorption (indicated by negative values inside brackets) in MIP and NIP solutions. Release back of quercetin molecules followed by decomposition was observed. Very low adsorption of quercetin at this temperature was observed in both MIP and NIP solutions owing to faster rate of degradation. The mechanism for quercetin degradation is complex and can be influenced by several factors including acid-base conditions, aqueous environment, present of nucleophiles like water, alcohol and the hydrophobicity of polymers [8,20,23-27]. In our case, water, methanol, acid-base conditions, temperature, imprinted polymers (nucleophiles and hydrophobicity) were all suggested to participate in the quercetin degradation.

**Chromatographic analysis:** HPLC chromatograms recorded at 280 and 370 nm at pH 2 and pH 6 after 24 h at 40 °C are shown in Fig. 6. The quercetin peak eluted at about 3.3 min and the peaks eluted between 1.6-2.5 min were attributed to oxidation products of quercetin. That is, the peaks observed in Fig. 6 blue chromatograms appearing at lower retention

time than quercetin were attributed to quercetin oxidation products [6,20]. These peaks were narrow and sharper in the blank solution (Fig. 6a,d). However, only one big-broad dominating peak was observed in MIP and NIP solutions observed at 280 nm at around 1.7 min. Fig. 6a,d clearly show that quercetin behaved differently in strongly acidic and slightly acidic conditions in aqueous/methanol solution. It can be observed that the peak of quercetin in Fig. 6a was lower than Fig. 6d. The difference in the peak shapes and retention times demonstrate that the presence of MIP and NIP particles influenced the decomposition mechanism of quercetin. It is known that quercetin easily undergoes oxidation in the presence of air oxygen in a rather wide pH range [8] and the stability of the intermediate oxidation products is largely dependent on the surroundings (organic or aqueous) offering a great consequential chemistry [23] and the oxidation products are best monitored in the UV range [6,20]. In our case the reactions were carried out in methanol-water (7:3 v/v) mixture and it can be deduced that this had an effect on the degradation of quercetin. Quercetin is poorly soluble in water hence most studies have used alcohol/water mixtures for studying the oxidation of quercetin with varying results [24-27]. The presence of water was found to act as a catalyst [24] for quercetin oxidation to *ortho*-quinones [25]. The broad peak observed after contacting quercetin solution with MIP or NIP particles at different pHs was attributed to *ortho*-quinone oxidation product. However, more detailed study on the separation and identification of the degradation products need to be done.

## Conclusion

Molecularly imprinted polymers and non-imprinted polymers were synthesized using precipitation polymerization. SEM images confirmed that MIP particles produced were monodispersed while FTIR showed that MIP and NIP had similar structural backbone due to strong characteristic bands at 1720 and 1110  $\text{cm}^{-1}$  assigned to the C=O and C-O bonds of the ethylene glycol dimethacrylate. Time, pH and temperature played a crucial role in the adsorption and degradation of quercetin. The highest degradation was observed at pH 2 at 60 °C after 5 days as determined by the HPLC. The unusual highest degradation of quercetin at pH 2 was attributed to complex quercetin interactions with the surrounding media. The extended exposure of quercetin in acid-alkaline conditions facilitated production of oxidation products. Therefore, in rebinding of quercetin by MIP, higher temperatures with extended adsorption times should be avoided since this may lead to decomposition of quercetin due to presence of nucleophiles in solution. The most promising parameters were achieved at lower temperature (20 °C) in which quercetin was adsorbed in substantial amounts with lower degradation. Adsorption and subsequently leaching of quercetin back into solution was observed and this demonstrated a very significant property that may be exploited for drug delivery systems.

## ACKNOWLEDGEMENTS

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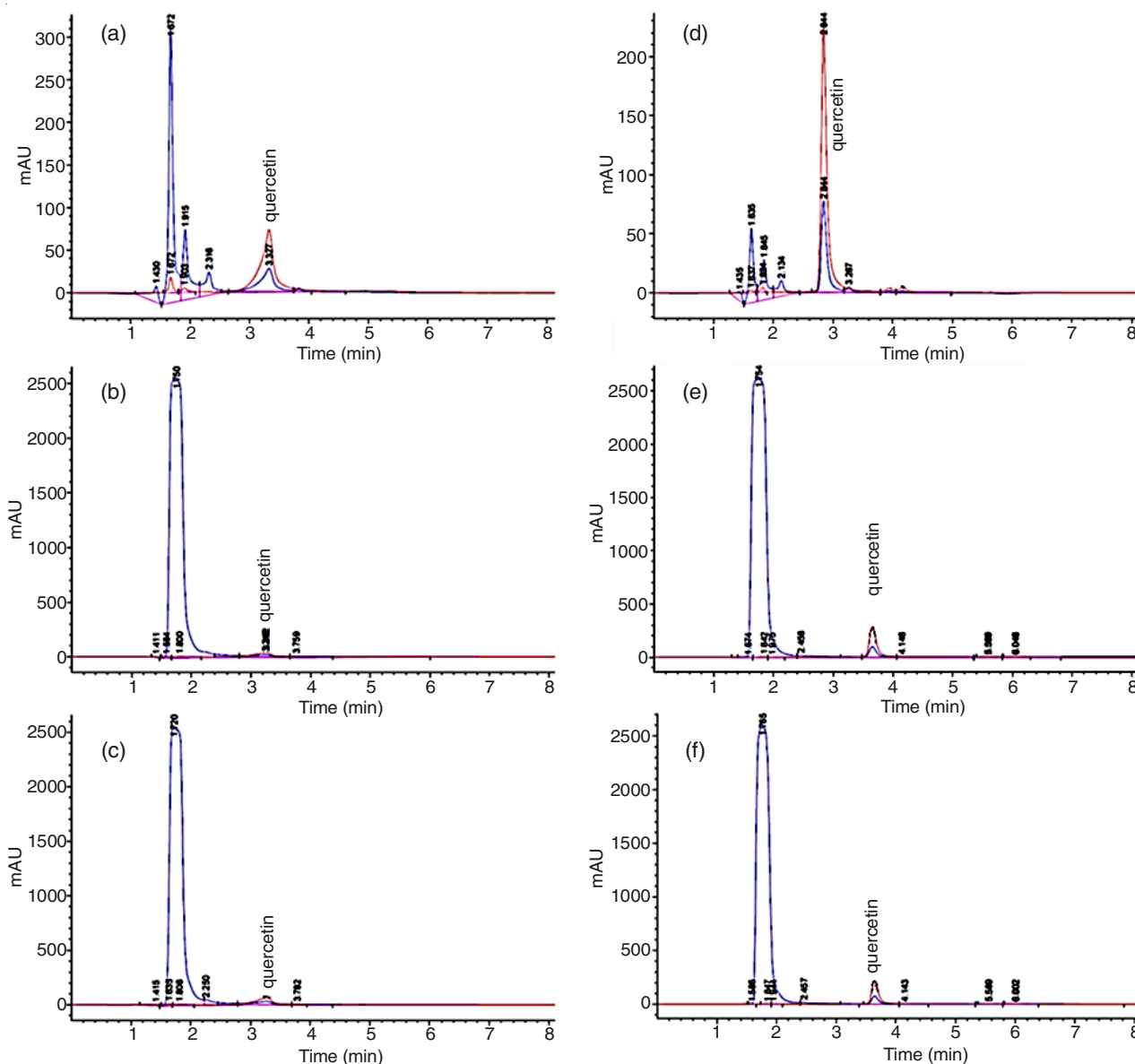


Fig. 6. HPLC chromatograms for blank solution (a), MIP solution (b) and NIP solution (c) at pH 2 after day 1 at 40 °C; for blank solution (d), MIP solution (e) and NIP solution (f) at pH 6 after day 1 at 40 °C. The blue chromatogram was recorded at 280 nm while the red was recorded at 370 nm

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