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Original Article

Analysis of residual crosslinking agent content in UV cross-linked poly(ethylene oxide) hydrogels for dermatological application by gas chromatography ☆

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ABSTRACT

Acrylates have been widely used in the synthesis of pharmaceutical polymers. The quantitation of residual acrylate monomers is vital as they are strong irritants and allergens, but after polymerization, are relatively inert, causing no irritation and allergies. Poly(ethylene oxide) (PEO) hydrogels were prepared using pentaerythritol tetra-acrylate (PETRA) as UV crosslinking agent. A simple, accurate, and robust quantitation method was developed based on gas chromatographic techniques (GC), which is suitable for routine analysis of residual PETRA monomers in these hydrogels. Unreacted PETRA was initially identified using gas chromatography–mass spectrometry (GC–MS). The quantitation of analyte was performed and validated using gas chromatography equipped with a flame ionization detector (GC–FID). A linear relationship was obtained over the range of 0.0002%–0.0450% (m/m) with a correlation coefficient (r^2) greater than 0.99. The recovery (> 90%), intra-day precision (%RSD < 0.67), inter-day precision (%RSD < 2.5%), and robustness (%RSD < 1.62%) of the method were within the acceptable values. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0001% (m/m) and 0.0002% (m/m), respectively. This assay provides a simple and quick way of screening for residual acrylate monomer in hydrogels.

1. Introduction

Hydrogels are cross-linked polymeric materials possessing the ability to swell and retain significant amount of water within their structure without dissolving. The biomedical applicability of hydrogels was first reported more than 50 years ago by Wichterle and Lim in their paper featuring poly(2-hydroxyethyl methacrylate) (pHEMA) gels as soft contact lenses [1]. However, it was not until the late 1980s that the feasibility of these materials as patches for dermatological [2] and transdermal [3] application was acknowledged. Recent research trends focused on the combination of hydrogels with delivery technologies such as nanotechnology, iontophoresis, and microneedle arrays to enhance skin penetration [4–6]. This ultimately widens the transdermal market, as the transportation of conventional hydrophobic small drug molecules, macromolecules and hydrophilic drug molecules has been made possible. Meanwhile, the development of hydrogel dressings and their potentials in wound healing as well as anti-scar activity is still an area of interest among researchers [7–9].

Synthetic polymer poly(ethylene oxide) (PEO) is clinically an ideal vehicle for topical and transdermal drug delivery due to little or no immunogenicity issues, absence of residues, sediments or vaporous elements when applied onto the skin. The biocompatibility and inertness of this FDA-approved polymer have led to the marketing of Vigilon®, which is a radiation crosslinked PEO hydrogel-wound dressings [10].

The fabrication of PEO hydrogels via gamma irradiation or electron beam (EB) has been well established [11,12] due to the ability of polymers to crosslink without any excipients. Later, chemical crosslinking of end groups in PEO chains with multifunctional crosslinking agents [13] led to the adoption of ultraviolet (UV) initiated crosslinking method as an alternative [14]. The advantages of UV irradiation technique are not restricted only to health and safety reasons, low cost, and efficient gel formation under a short period of time; UV cross-linked PEO hydrogel films showed significantly stronger mechanical profiles than pure PEO films obtained via EB, with Young's moduli similar to those of human skin [15].

The crosslinking of PEO hydrogels via UV radiation was initially performed in the presence of free radical initiators, resulting in

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synthesized gels containing low molecular weight aromatic impurities. Doytcheva et al. [14] tackled this issue by direct UV crosslinking of PEO chains in the presence of multifunctional acrylate monomers as crosslinking agents. Despite such breakthrough procedure, the amount of residual monomer present in the resultant films has not been evaluated. This is of great importance in the production and quality control of PEO films intended for dermatological application, as unreacted acrylate monomers can potentially leach out from the films, causing side effects such as inflammation and skin sensitization.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the two most commonly employed methods in the analysis of residual monomer, because of the simplicity and rapidity of these techniques [16]. The preferred method is solely dependent on the volatility and water solubility of the monomer itself. Other methods that have been used to quantify residual monomers involved estimation of double bonds either by chemical methods such as bromination or spectroscopic techniques [17–19].

Based on the existing literature, the amount of residual acrylate monomers in hydrogels has been previously determined by Ende et al. using GC [20], and Snezana et al. using HPLC [21]. Both techniques require an extraction process prior to analysis. However, none of these has been validated to ensure dermatological safety. Therefore, the aim of this work was to present a simple and rapid analytical procedure for accurate quantitation of residual acrylate monomer in hydrogels by GC as a quality control test for hydrogel films for skin application.

2. Experimental

2.1. Materials

Poly(ethylene oxide) (PEO) ($\bar{M}m = 1000{,}000$ g/mol), pentaerythritol tetraacrylate (PETRA), hexylacrylate (HA), and dichloromethane (DCM) were purchased from Sigma-Aldrich (Dorset, UK). All materials were used as received. Distilled water (Triple Red, Long Credon, UK) was used for dissolving PEO and swelling of PEO cross-linked films. Unmedicated PEO hydrogel films were synthesized via UV crosslinking with varying concentrations of multifunctional monomer pentaerythritol tetra-acrylate (PETRA) as cross-linking agent, as explained in the literature [15,22].

2.2. Instrumentation and chromatographic conditions

2.2.1. Identification of residual PETRA

The residual PETRA content was initially identified using a gas chromatograph mass spectrometer (GC–MS). The GC–MS analysis was performed using an Agilent Technologies 7890A Gas Chromatograph (Agilent Technologies, Santa Clara, California, USA) coupled to an Agilent Technologies 5975 C MSD (Mass Selective Detector) with Triple-Axis Detector (Agilent Technologies, Santa Clara, California, USA) in full scan mode, scanning from 40 to 200 mass to charge ratio (m/z). Chromatographic separation was achieved on a fused-silica capillary column (30 m × 0.25 mm × 0.25 μm) coated with 5% phenyl-methylpolysiloxane. The injector was in splitless mode and its temperature was maintained at 300 °C throughout the experiments. The column temperature was raised from 50 °C (1 min hold time) to 280 °C (4 min hold time) at a rate of 30 °C/min. The flow rate of carrier gas (helium) was 2 mL/min. The identification of compounds was carried out by comparing the full scan spectra with spectra obtained from literature.

2.2.2. Quantitation of residual PETRA

The quantitation of residual PETRA was carried out and validated using an Agilent Technologies 7820A Gas Chromatograph equipped with a flame ionization detector (GC–FID) (Agilent Technologies, Santa Clara, California, USA). Chromatographic separation was achieved on a fused-silica capillary column (30 m × 0.25 mm × 0.25 μm) coated with 5% phenyl-methylpolysiloxane. The injector was in splitless mode and its temperature was maintained at 300 °C throughout the experiments. The column temperature was raised from 50 °C (1 min hold time) to 280 °C (4 min hold time) at a rate of 30 °C/min. The flow rate of carrier gas (nitrogen) was 6.34 mL/min.

2.2.3. Internal standard solution

HA was used as an internal standard to compensate for loss of analyte during sample preparation and instrumental analysis. In order to achieve an internal standard peak area representing analyte concentration at the midpoint of the calibration curve, 100 mg of HA was accurately weighed and diluted with dichloromethane (DCM) to a total volume of 100 mL. The final concentration of the internal solution was 1 mg/mL, which was approximately equivalent to 0.075% (m/m). The internal standard solution was then included in all standard and sample solutions.

2.2.4. Standard solution and calibration curve

A PETRA stock solution with a final concentration of 900 μg/mL was prepared by dissolving adequate amount of compound in DCM. Seven standard solutions with PETRA concentrations of 0.0002%, 0.0004%, 0.0017%, 0.0056%, 0.0226%, 0.0339% and 0.0451% (m/m) (2.4, 5.6, 22.5, 75, 300, 450, and 600 μg/mL) were prepared by pipetting volumes of stock solution into respective 50 mL volumetric flasks, along with 2.5 mL of internal standard solution and diluting to volume with solvent. Triplicate measurements were carried out at each concentration. A calibration curve was obtained by plotting the peak area ratio of PETRA to the peak area of HA against the corresponding PETRA concentration. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated based on a signal-to-noise ratio (S/N). It is generally accepted that the concentration that generates a peak with S/N value of 3 is regarded as LOD [23]. On the other hand, LOQ was estimated based on the lowest concentration used to obtain a sharp and symmetrical peak, which resolves within 10% of the baseline [24]. For confirmation, five PETRA standards with concentrations corresponding to LOD and LOQ were prepared for measurements.

2.3. Sample preparation

Four replicates of dry hydrogel films weighing approximately 0.1 g were immersed in distilled water for 72 h at 25 °C in order to wash out uncrosslinked PEO. The swollen gels were dried in a vacuum oven at 40 °C. The dried gels (xerogels) were then individually cut into small pieces, placed in a glass vial and extracted with 10 mL of DCM for 24 h at room temperature. During extraction, the tightly closed vials were manually shaken for at least five times. After that, 0.5 mL of internal standard solution was added into the hydrogel extracting medium and 1 μL of the resultant mixture was directly injected into the GC for analysis. All samples were analysed in triplicate.

2.4. Data acquisition and processing

The Agilent Mass Hunter version B.05.00 software was used for identification of residual PETRA monomer peak and Agilent ChemStation B.04.03 software was used to calibrate and quantify the responses of residual PETRA monomer.

3. Results and discussion

3.1. PETRA monomer

3.1.1. Physicochemical properties of PETRA

PETRA is a fast curing, tetra-functional monomer. The colourless viscous liquid is mainly used as a crosslinking agent in UV/EB initiated polymerization. Some of the physicochemical properties of PETRA [25–27] are summarized in Table 1.

3.1.2. Toxicology of PETRA

According to the Global Harmonization System (GHS) classification, PETRA is a Category 1 skin sensitizer; evidence of skin sensitization was found during patch testing in human and in guinea pig maximization test [28]. Since data regarding the maximum acceptable concentration of PETRA residual monomer was not available in literature, we determined the concentration threshold to be 0.0100% (m/v), based on the lowest challenge concentration of pentaerythritotol triacrylate (PETA) that elicited a positive skin sensitization response in humans [29]. PETA is a monomer similar to PETRA in both structural and chemical properties. It should be noted that PETA is a stronger sensitizer than PETRA [30]. The maximum acceptable residual PETRA concentration was recalculated based on the density of PEO (1.21 g/mL), and was found to be 0.0126% (m/m).

3.2. Method development

The proposed sample extraction method was adapted from Ende et al. [20], but using a different solvent as sample extracting medium instead of water, due to the fact that PETRA is water immiscible (Table 1). For this reason, the development of our GC method was started with the selection of a suitable solvent as the extracting medium. This is important as the extraction efficiency is largely dependent on the type of solvent used [31]. The suitable solvent needs to be volatile to dissolve HA and PETRA and at the same time be able to swell the PEO films. Acetone and DCM fulfilled these criteria, but PEO films were able to swell to a much greater extent in DCM [32]. Hence, DCM was selected as the extracting medium.

3.3. GC–MS identification of PETRA

Fig. 1 illustrates the GC–MS spectra of fragment ions of internal standard HA (MW=156.22 g/mol) and PETRA (MW =352.12 g/mol) obtained from literature compared to our sample assays. The mass spectra from our assay were very similar to the mass spectra in the literature [27] for both compounds. HA is an appropriate internal standard in the assay of PETRA, as its mass spectrum is similar but not identical to PETRA, and it does not co-elute with the analyte of interest. Both HA and PETRA did not exhibit a molecular ion peak in their spectra but showed dominant fragment, $m/z=55$, which corresponded to the acryloyl ion ($Acr^+ \equiv [H_2C=CH\text{-}C\equiv O]^+$) [33]. This acryloyl ion peak is typical for acrylates. The spectrum of PETRA also showed characteristic fragments, $m/z=81(C_6H_9^+)$ [34] and 126 ($[H_2C=CH\text{-}C\text{-}O\text{-}O\text{-}CH_2\text{-}CH\text{-}C\equiv O]^+$) [33].

3.4. Gas chromatography analysis

The optimal condition for the determination of residual PETRA was investigated. Under the conditions mentioned in Section 2.2, gas chromatograms of HA and PETRA were well resolved, and had good peak separations. This indicates that the test method is selective and specific in determining residual PETRA in PEO hydrogels. Typical gas chromatograms of standard and sample are presented in Fig. 2. Since there were three peak fractions (peak a, b, and c) corresponding to PETRA on the gas chromatograms, only the most abundant peak (peak b) was used for analysis. The retention time for HA and PETRA was slightly different when analyzed by GC–MS and GC–FID as different carrier gases were supplied. When analyzed by GC–MS, the average retention times of HA and PETRA was 4.170 ± 0.001 and 8.227 ± 0.006 min, respectively ($n=10$), while GC–FID analysis gave average retention time of 4.410 ± 0.001 and 9.001 ± 0.008 min, respectively ($n=10$).

3.5. GC–FID quantitation of residual PETRA

The developed quantitation method was validated for linearity, accuracy, precision, sensitivity (LOD and LOQ) and robustness.

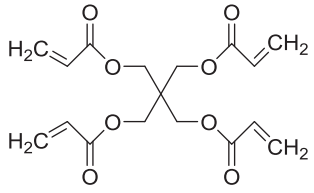
3.5.1. Linearity and range

A calibration curve of peak area ratio vs. PETRA concentration was obtained from seven PETRA standards with concentrations ranging from 0.0002% to 0.0451% (m/m) as explained in Section 2.2.4. The collected data were average values of three injections of each concentration. A good linear relationship was obtained ($y=43.228x-0.012$, correlation coefficient (r^2)=0.99886), with ranges comfortably covering the threshold limit of residual PETRA. These features confirmed the feasibility of calibration plot for assessing the dermatological safety of manufactured PEO films.

3.5.2. Accuracy

The accuracy of the proposed extraction method was determined by carrying out extraction on samples spiked with known quantities of PETRA monomer and on samples without monomer spiking (blank). Spiked samples were prepared in triplicate using three PETRA concentration levels. The amount of residual monomer measured in the unspiked sample was used as a reference. Triplicate measurements were carried out in all samples (weighing approximately 0.1000 g). The percentage recoveries

Table 1
Physicochemical properties of PETRA.

Molecular structure	CAS number	Molecular weight (g/mol)[a]	Solubility in water [b]	Melting point (°C)[a]	Boiling point (°C)[c]
	4986–89-4	352.34	Immiscible	18	> >220

[a] Taken from Chemical Book [25].
[b] Taken from Santa Cruz Biotechnology [26].
[c] Taken from Unwin [27].

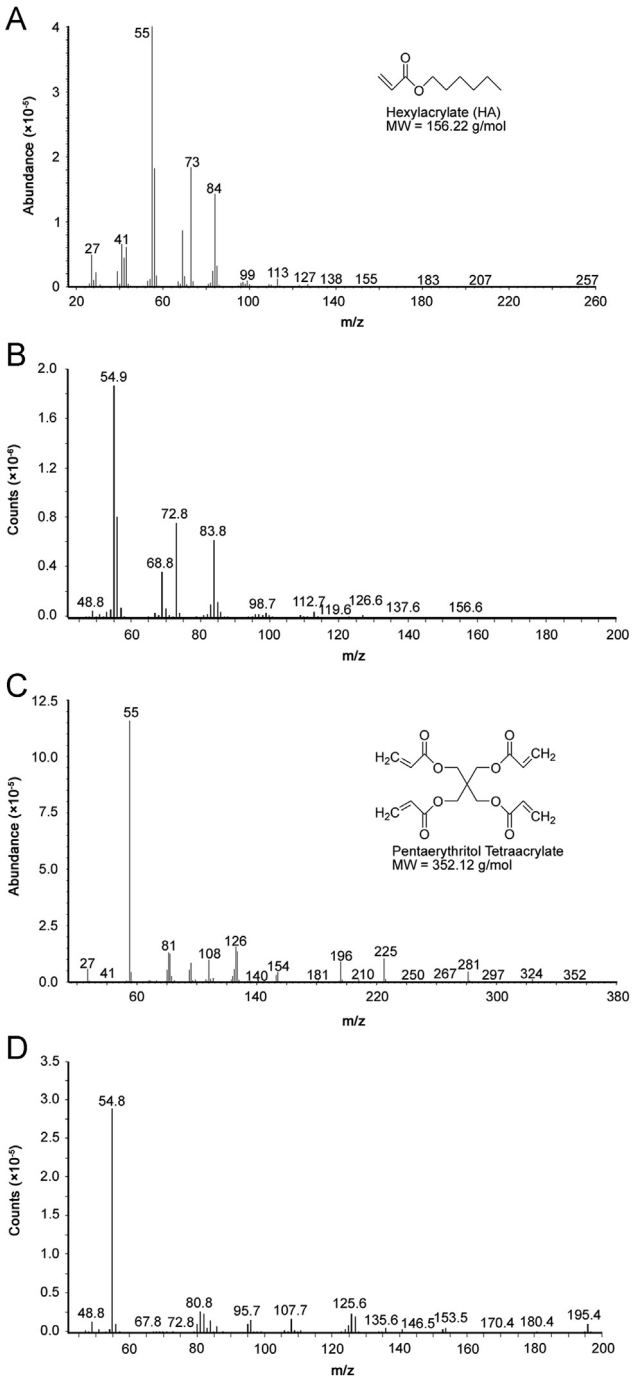


Fig. 1. (A) Mass spectrum of HA adapted from Unwin [27]; (B) sample scan mass spectrum of HA; (C) mass spectrum of PETRA adapted from Unwin [27]; (D) scan mass spectrum of PETRA in PEO hydrogel film.

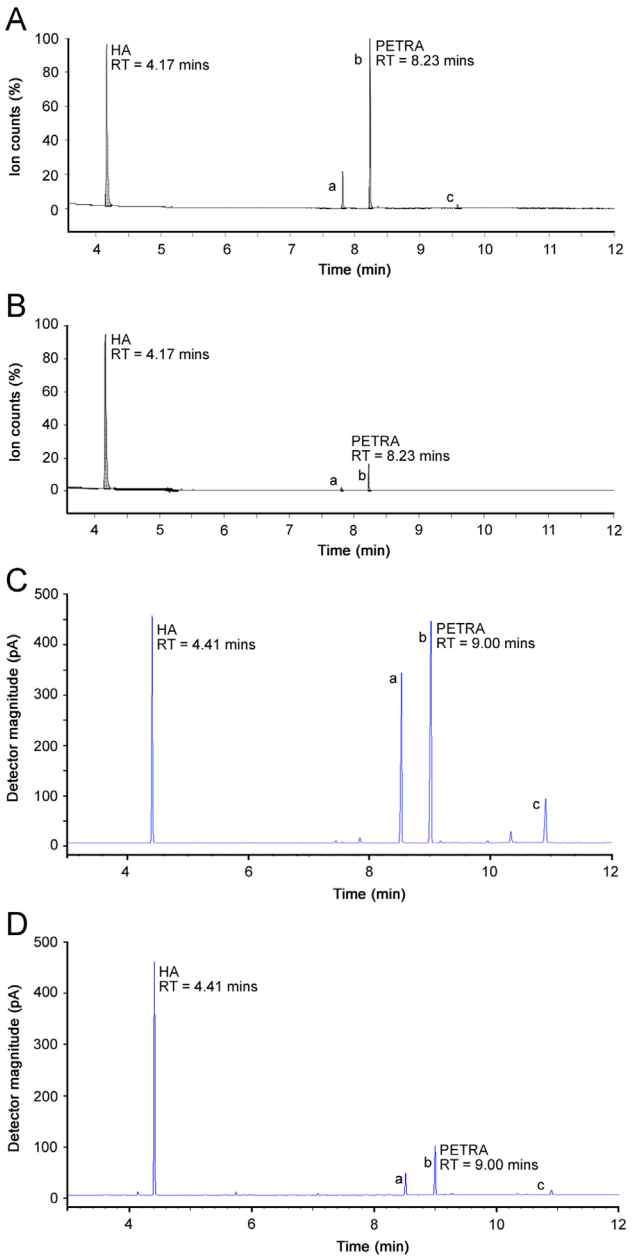


Fig. 2. Typical chromatograms of PETRA using HA as internal standard. (A) From a standard solution (450 μg/mL PETRA) by GC–MS; (B) from a sample solution of residual PETRA extracted from PEO film using GC–MS; (C) from a standard solution (450 μg/mL PETRA) using GC–FID; and (D) from a sample solution of residual PETRA extracted from PEO film using GC–FID.

were calculated as follows:

$$Accuracy\,(Recovery\,(\%)) = \frac{C_{recovered}}{C_{spiked} + C_{blank}} \times 100\%$$

Where $C_{recovered}$ is the concentration of PETRA monomer measured in the spiked samples (%, m/m), C_{spiked} is the concentration of PETRA added to spiked samples (%, m/m) and C_{blank} is the concentration of PETRA monomer measured in the blank samples (%, m/m).

The accuracy results (Table 2) showed that the extraction method was suitable for the accurate quantification of residual PETRA in PEO hydrogels, with an average recovery of 92.20% (within 80%–120%) and relative standard deviation (%RSD) of 2.32% (within ± 15%). In addition, the recovery values were also consistent for all concentrations tested. Although higher efficiencies may be achieved by other techniques such as Soxhlet extraction and microwave-assisted extraction, the simplicity and ease of implementation of the proposed method makes it advantageous for routine analysis of residual multifunctional acrylate monomer in hydrogels.

3.5.3. Precision

The precision of the quantitation method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). The intra-day precision was evaluated by performing triplicate measurements at three different PETRA concentration levels on three occasions of the same day. As for

Table 2
Accuracy results of sample extraction at different PETRA concentrations ($n=3$).

Concentration added (%, m/m)	Concentration in blank (%, m/m) (mean ± SD)	Concentration recovered (%, m/m) (mean ± SD)	Mean recovery (%)	RSD (%)
0	0.0019 ± 0.0004			
0.0075		0.0063 ± 0.0001	91.14 ± 1.38	1.51
0.0015		0.0155 ± 0.0004	92.49 ± 2.37	2.56
0.0298		0.0277 ± 0.0008	92.96 ± 2.67	2.88
Mean			92.20	2.32

Table 3
Intra- and inter-day precision of residual PETRA.

PETRA concentration (%, m/m)	Intra-day precision ($n=3$)		Inter-day precision ($n=9$)	
	Peak area ratio (mean ± SD)	RSD (%)	Peak area ratio (mean ± SD)	RSD (%)
0.0056	0.204 ± 0.000	0.20	0.204 ± 0.005	2.49
0.0226	0.942 ± 0.006	0.67	0.961 ± 0.011	1.15
0.0339	1.422 ± 0.007	0.52	1.447 ± 0.023	1.59
Mean		0.46		1.74

Table 4
LOD and LOQ of PETRA.

Parameter	Concentration (%, m/m)	Signal to noise ratio ($n=5$) (mean ± SD)
Limit of detection (LOD)	0.0001	3.4 ± 0.23
Limit of quantitation (LOQ)	0.0002	10.2 ± 0.62

inter-day precision, the analysis was carried out in a similar manner to that of intra-day precision for three consecutive days. In order to maintain data consistency, all precision results were determined in the same laboratory, by the same operator, and using the same equipment. Both inter- and intra-day precision results are tabulated in Table 3. From the data obtained, the developed GC method was concluded as precise, with mean intra- and inter-day precision (%RSD) of 0.46% and 1.74%, respectively. The high degree of precision indicated the method is repeatable.

3.5.4. Sensitivity

The sensitivity of quantitation was assessed by LOD and LOQ. During the assay, only sharp symmetrical peaks were considered. Peaks that appeared to be excessively broad, or those showing tailings or shoulders were rejected. The sensitivity results are tabulated in Table 4. It was observed that the concentration corresponding to LOQ was about 0.0001% (m/m), whereas the concentration found representing LOQ was about 0.0002% (m/m). This finding is in line with the statement of LOQ being typically two times higher than LOQ [35]. As low values were obtained, the method was concluded as highly sensitive.

3.5.5. Robustness

Robustness study was conducted to evaluate the method's reliability during normal usage. The proposed method was challenged by the following parameters: initial oven temperature 50 ± 5 °C, detector temperature 300 ± 5 °C and flow rate ± 10%. Three levels of PETRA concentrations were measured in triplicate for each set of variation. The acceptance criteria were set as RSD values not exceeding 10% for each concentration level and a maximum cumulative (overall) RSD of 15% [36]. The obtained results are summarized in Table 5. The peak area ratios for all concentrations studied were highly reproducible, with corresponding RSD values (0.20%–1.62%) well within the acceptance criteria. The method was therefore confirmed to be robust.

Table 5
Robustness results at different levels of PETRA concentration.

Varying conditions	PETRA concentration (%, m/m)	Peak area ratio (%) ($n=3$) (mean ± SD)	RSD (%)
No variation	0.0056	0.204 ± 0.000	0.20
	0.0226	0.942 ± 0.006	0.67
	0.0339	1.422 ± 0.007	0.52
Oven temperature (+5 °C)	0.0056	0.204 ± 0.001	0.23
	0.0226	0.946 ± 0.005	0.72
	0.0339	1.420 ± 0.013	0.81
Oven temperature (−5 °C)	0.0056	0.205 ± 0.001	0.54
	0.0226	0.957 ± 0.006	1.16
	0.0339	1.424 ± 0.16	1.12
Detector temperature (+5 °C)	0.0056	0.204 ± 0.001	0.27
	0.0226	0.944 ± 0.003	0.53
	0.0339	1.405 ± 0.008	0.77
Detector temperature (−5 °C)	0.0056	0.204 ± 0.001	0.63
	0.0226	0.967 ± 0.006	1.60
	0.0339	1.450 ± 0.006	0.91
Flow rate (+10%)	0.0056	0.205 ± 0.001	0.29
	0.0226	0.096 ± 0.011	1.62
	0.0339	1.430 ± 0.021	1.28
Flow rate (−10%)	0.0056	0.021 ± 0.001	0.37
	0.0226	0.097 ± 0.008	1.60
	0.0339	1.414 ± 0.025	1.54
Mean			0.83

3.6. Method application

The effect of PETRA content on the amount of residual PETRA present in PEO hydrogel films was determined and is summarized in Table 6. The acceptable limit for residual PETRA in the films was below 0.0126% (m/m), as explained in 3.1.2. All film samples prepared with a maximum PETRA concentration of 7.5% (m/m) passed the residual PETRA test. Films failing to meet the established criteria were first identified in those prepared with 10% (m/m) PETRA. Therefore, the maximum acceptable PETRA concentration limit in producing PEO hydrogel films that are safe for dermatological application was found to be 10% (m/m).

4. Conclusion

A GC method was developed for the analysis of residual PETRA monomers in PEO hydrogels. The quantitation method was validated and proven to be accurate, precise, sensitive and robust. Furthermore, the simple sample preparation and quick assay (total run time of 12 min) indicated that the method is suitable for routine analysis of residual acrylate monomer in hydrogels. The maximum acceptable PETRA concentration limit in producing PEO hydrogel films that are safe for dermatological application is concluded as 10% (m/m).

Table 6
Effect of PETRA concentration on the amount of residual PETRA.

PETRA concentration (%, m/m)	Sample	Mean residual PETRA concentration (%, m/m)	Pass/fail residual PETRA% < 0.0126% (m/m)
1	1	Not detectable	Pass
	2	Not detectable	Pass
	3	Not detectable	Pass
	4	Not detectable	Pass
Mean	–	–	Pass
2.5	1	< 0.0124[a]	Pass
	2	< 0.0117[a]	Pass
	3	Not detectable	Pass
	4	Not detectable	Pass
Mean	–	–	Pass
5	1	< 0.0105[a]	Pass
	2	< 0.0120[a]	Pass
	3	< 0.0111[a]	Pass
	4	< 0.0123[a]	Pass
Mean		< 0.0114 (± 0.0007)	Pass
7.5	1	< 0.0119[a]	Pass
	2	< 0.0121[a]	Pass
	3	< 0.0118[a]	Pass
	4	< 0.0119[a]	Pass
Mean		< 0.0118 (± 0.0001)	Pass
10	1	< 0.0128[a]	Fail
	2	< 0.0106[a]	Pass
	3	< 0.0121[a]	Pass
	4	< 0.0112[a]	Pass
Mean		< 0.0117 (± 0.0009)	Pass
20	1	< 0.0239[b]	Fail
	2	< 0.0250[b]	Fail
	3	< 0.0132[a]	Fail
	4	< 0.0113[a]	Pass
Mean		< 0.0184 (± 0.0063)	Fail
40	1	1.0943 (± 0.0183)	Fail
	2	2.6219 (± 0.0493)	Fail
	3	0.5697 (± 0.0038)	Fail
	4	0.1375 (± 0.0086)	Fail
Mean		1.148 (± 0.8661)	Fail

[a] < LOD;
[b] < LOQ.
Values in brackets indicate the standard deviation (SD) from the reported mean.

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