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Evaluating thermogravimetric analysis for the measurement of drug loading in mesoporous silica nanoparticles (MSNs)

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ABSTRACT

In this study, a thermogravimetric analysis (TGA) method for measuring the drug loading in mesoporous silica nanoparticles (MSNs) has been developed and evaluated in comparison with the drug loading quantification by high-performance liquid chromatography (HPLC). Indapamide was loaded into two different types of MSNs, namely Mobile Crystalline Material (MCM-41, pore size = 1.2 nm) and Santa Barbara Amorphous (SBA-15, pore size = 4.1 nm). Physical mixtures of the drug and silica gave a linear correlation between the observed and expected drug content for both TGA and HPLC, which were used for calibration purposes. The limit of detection (LOD) for the TGA method obtained from the physical mixture calibration curve was 0.77 % (w/w) and the r^2 value was 0.9936, whereas the HPLC had a LOD of 0.06 % (w/w) and an r^2 value of 0.9933. The sensitivity of the TGA method was well established using the drug loading studies, as it can detect the low loading of MCM-41 at 2.2 ± 0.21 % (w/w), compared to 5.1 ± 0.12 % (w/w) with the SBA-15. In all samples applied, the multiple comparison analysis showed an insignificant difference between the two methods ($p > 0.05$). The TGA data presented good evidence for using this technique as a sensitive, cost-effective, and low-variable quantitative analysis in the drug loading determination of the MSNs. TGA is not a selective method of quantification, but optimising the method using the pure and blank samples of MSNs and drug can significantly improve the sensitivity. This work provides a unique approach to apply TGA as a selective and more favourable method to characterise MSNs to do early formulation developments.

1. Introduction

Mesoporous silica nanoparticles (MSNs) are a smart platform for many drug delivery applications [1]. They feature high loading due to their ordered pore structure, chemical stability, tunable pore diameter (2–10 nm), high pore volume (~ 1 cm^3 /g), and high surface area (800 m^2/g) [2–7]. However, the analysis techniques applied to measure the loading of silica are associated with challenges and some disadvantages. High-performance liquid chromatography (HPLC) is the most common analytical technique used in the measurement of the drug loading into MSNs. It can be used indirectly to quantify the amount of unentrapped drug in the loading medium [8,9]. In such studies, the drug loading is often carried out using the adsorption method, which involves mixing a high concentration of drug solution with solid particles of silica for specific amounts of time [8,9]. The drug loading is then calculated by subtracting the amount detected in the loading solution from the initial drug mass. This measurement assumes that all undetected drug was incorporated into the silica particles; such an assumption may lead to complexity in the interpretation because the drug may have potentially absorbed onto the wall of the loading container, which is not accounted for in calculation. This contribution however will be relatively small. Also, this measurement is not suitable in the case of drug loading carried out using a minimum of solvent, which cannot be separated from the particles, such as when the drug is loaded using the incipient wetness impregnation method [10]. An alternative measurement of drug loading may be carried out by extracting the loaded drug using a suitable solvent and then using HPLC to quantify the drug in the extraction solution [11, 12]. Although this method is described in the literature as a

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conventional procedure, it can be time-consuming because the extraction method must be optimised to achieve the best drug recovery. MSNs have a porous structure that can retain compounds deeply in their pores; consequently, enough of a concentration gradient should be applied to extract all the incorporated drugs. The adsorption method of drug loading quantification is a multi-step process of extraction, separation, and analysis that can take several hours and is often associated with many variables [13]. Additionally, the total cost of applying this method should be considered, as many solvents are often needed, including expensive HPLC grade solvents [14].

Thermogravimetric analysis (TGA) has the potential to provide a relatively rapid measurement of drug loading in a single-step process [15]. It has the advantages of ease of sample preparation and being less costly to perform the experiments compared to HPLC [16,17]. In the energy industry, TGA has emerged as an alternative, less expensive, fast, and easy to use the technique for determining the composition of the lignocellulosic biomass, compared to the commonly used wet chemical techniques [18]. The TGA technique has already been successfully used in the drug loading measurements of the MSNs [19–23]. Notwithstanding the successful application of TGA for drug loading determination, there is often insufficient information concerning methodology and method development to allow routine application of this technique. It is worth noting that most of the TGA data reported with regards to drug loading has very little information regarding the experimental error and the variance in the observed values for drug loading. There is doubt about the sensitivity and selectivity of the technique in determining the loading of nanoparticles [24]. The thermal decomposition of the drug is often detected as a temperature-dependent mass loss [24–26]. TGA measurements have been reported to be dependent not just on the drug loading but also on the interaction of the degradation products with the carrier [27]. Depending on the specific mechanism and volatility of the products, such an interaction may lead either to an underestimation or an overestimation of the drug loading. Thus, optimised TGA methodologies and detailed interrogation of the thermograms are required. Furthermore, the measurements can be affected by the presence of volatile components such as organic solvents and hydrates in the samples, which may overestimate the measured values [28].

Interestingly, MSNs are highly stable inorganic materials that offer a perfect distinction in the thermogram between low- and high-temperature mass loss events and concomitant breakdown [29]. As a result, a new approach is required to optimise the method of drug loading by employing TGA in order to precisely estimate drug content and its associated mass loss. This study aims to evaluate thermogravimetric analysis for the measurement of drug loading in mesoporous silica nanoparticles (MSNs) in comparison to HPLC.

2. Materials and methods

The two types of MSNs were purchased from Sigma Aldrich; the Mobile Composite Matter No. 41 (MCM-41) (batch no: MKCD5902), and the Santa Barbera Amorphous (SBA-15) (batch no: MKCG3359). Indapamide was obtained from Sigma (batch no. BCCD6339).

2.1. Morphology and surface analysis of MSNs

The particle size and morphology of the silica samples were examined by the Zeiss Auriga FIB/SEM XBeam system, which is equipped with a Cobra-focused gallium ion beam column, a Schottky field emission gun, and a Gemini electron column. ImageJ software was used to do the particle size measurements. The nitrogen adsorption experiments were conducted at liquid nitrogen temperature (−196 °C) on a Micromeritics Accelerated Surface Area and Porosimetry 2020 instrument in static mode. The samples were heated at a rate of 5 °C/min and outgassed under a high vacuum at 50 °C for 15 h before the sorption measurements. The Brunauer-Emmett-Teller (BET) equation was used to calculate the specific surface area from the adsorption data in the P/P_0 range between 0.1 and 0.3 [30,31]. On the desorption branch of the isotherm, the Barrett-Joyner-Halenda (BJH) method was used to determine the pore volume and the size distribution of the pores.

2.2. Interpretation of the TGA thermograms

To obtain the best result using TGA, the robustness of the system was checked by running an empty platinum pan from 25 °C to 900 °C at a heating rate of 10 °C/min. Plotting the mass loss (μg) allowed an investigation of drift in the system. Subsequently, the silica- and IND-as-received samples were run to calculate the mass loss of each. Next, the silica blank was run, which used the silica as-received sample soaked with ethanol (puriss. p.a., absolute, ≥ 99.8 %) to mimic the actual drug loading of the MSNs. This was performed to see the effect of the solvent on TGA thermograms. Finally, to better identify the area of drug decomposition, three different drug loads of silica (low, medium, and high) were prepared to be run in the TGA. The adsorption method of drug loading was explained below, but an excessive amount of drug was used in the loading solution. The mass ratios of silica to the drug that was used were 1:2.2, 1:2.3, and 1:2.5 (w/w). For all samples investigated, the weight losses (% w/w) observed in the TGA thermograms were calculated by converting the thermograms into their 1st derivative using the TA Universal Analysis Software.

2.3. Physical mixture sample preparation and calibration curve

The physical mixtures were prepared and analysed to study the sensitivity of the TGA method with respect to HPLC. Physical mixtures of commercial silica (MCM-41) and IND were prepared at different drug to silica ratios: 90, 75, 50, 25, and 10 % (w/w). In a 7 mL glass vial, 10 mg of each material was accurately weighed and mixed using a vortex mixture at 3000 rpm for 2 min. Each physical mix prepared was divided into two parts to be analysed using HPLC and TGA. With HPLC analysis, 5 mL of ethanol (puriss. p.a., absolute, ≥ 99.8 %) was used to extract the drug from the physical mixture. The suspensions were vortexed for 2 min at 3000 rpm, filtered using a syringe filter (0.45 μm, sterile PES filter), diluted, and analysed by the HPLC. The TG analysis was performed immediately for the second part of the physical mixtures. The calibration curve of the amount of drug detected using both techniques was plotted against the initial drug content in the physical mixtures, and all results were compared. The limit of detection (LOD) of both methods is calculated using the limit of blank (LOB) as presented in Eqs. (1) and 2, where the blank of HPLC analysis is the silica dissolved in ethanol and the blank of TGA is the total weight loss (%) of the silica as-received samples [15,32,33].

$$LOD = LOB + 1.645\ (standard\ deviation\ of\ low\ concentration\ sample) \quad (1)$$

$$LOB = mean\ blank + 1.645\ (standard\ deviation\ blank) \quad (2)$$

2.4. Assessing the uniformity of the physical mixtures

The uniformity of the physical mixtures prepared was investigated by applying the US dosage unit study based on the US pharmacopoeia of dosage units [34]. The test acceptance value (AV) was calculated using the following formula:

$$AV = [M - X] + ks \quad (3)$$

where M is the reference value, X is the sample mean, k is the acceptability constant, and s is the sample standard deviation. A sample of 5 mg was obtained from the physical mixture of the lower drug content (10 %). Then, 2 mL of ethanol was used to extract drugs from the mixture by applying a vortex at 13,000 rpm for 1 min, followed by the ethanol solution of IND being separated using centrifugation at 13,000 rpm for 15 min. The supernatant was collected, diluted, and analysed by

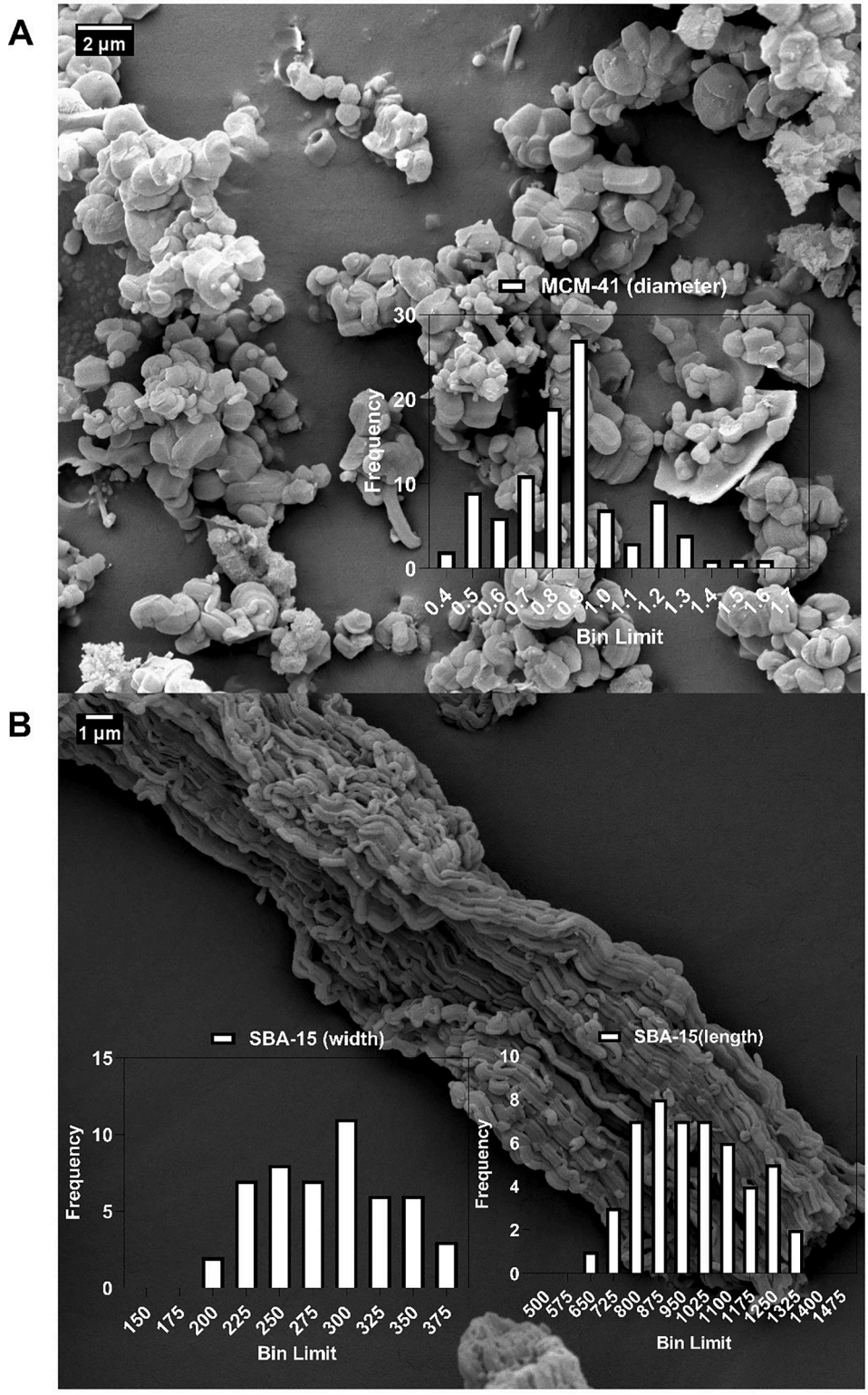

Fig. 1. The SEM for the MCM-41 (A) and SBA-15 (B) and their size distributions. The ImageJ software was used to obtain values of the MCM-41 diameter and SBA-15 length and width; 100 measurements were performed for each one. A gold coating was applied to the sample slides just before the SEM analysis. Each sample was prepared by making a suspension in EtOH (1 mg/mL), sonicating for 10 min, and then evaporating on microscope slides. The x axis represents the size measured using the ImageJ software. The unit of the bin limit is micron.

the HPLC.

2.5. The adsorption method of drug loading into MSNs

IND loading into MSNs was carried out using the adsorption method as reported in previous work, with some modifications [12]. Ethanol was used as a loading solvent because it is safe, not toxic, and can dissolve a large amount of drug. The MSN to drug ratio used was 1:1 (w/w). The resultant mixture was sonicated in a capped glass vial for 5 min using a Fisher Scientific (FS30D) bath sonicator at a frequency of 42

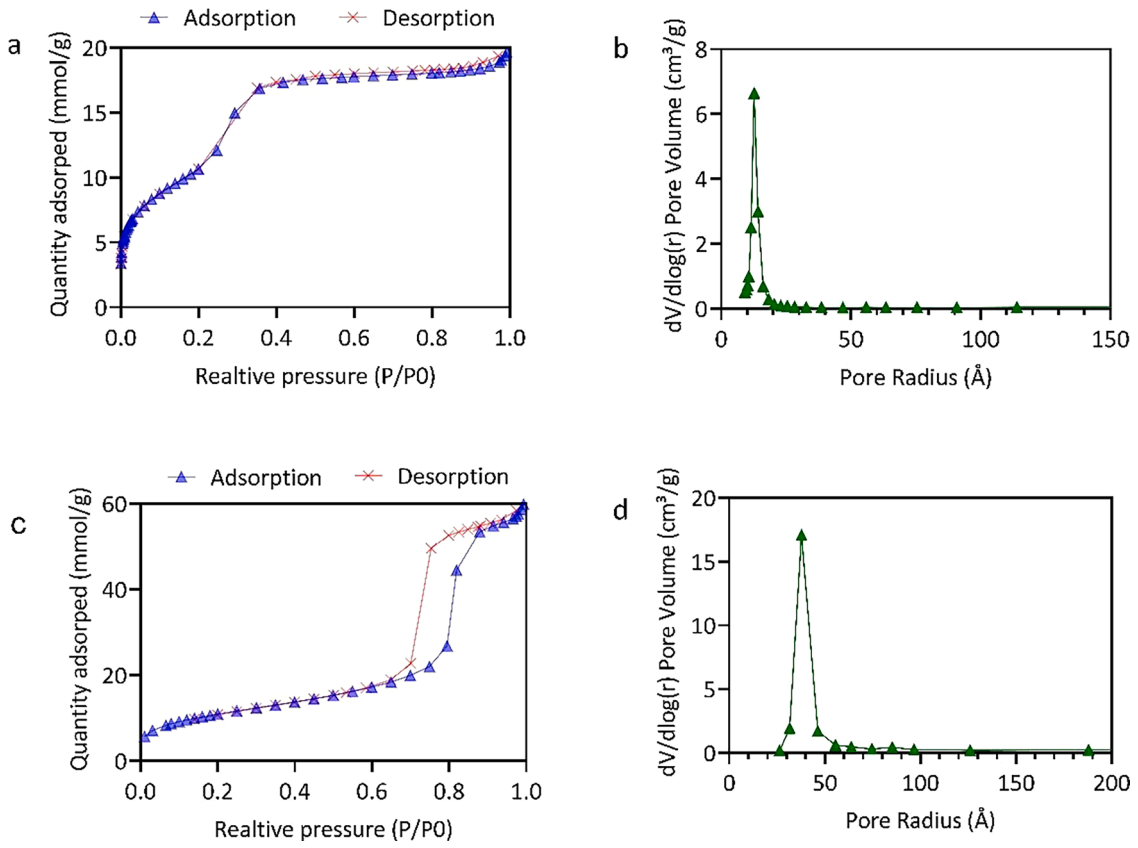

Fig. 2. The nitrogen sorption isotherms for MCM-41 (A) and SBA-15(C), as well as their corresponding pore size distributions (B and D). The pore size measurements obtained from the BJH method on the desorption path of the isotherm. A standard temperature and pressure were applied.

kHz and a power of 100 W. Afterward, the mixture was brought to adsorption equilibrium by gentle magnetic stirring for 24 h at 40 °C in the dark to achieve maximum penetration of IND into the pores channel. The loaded silica was recovered by centrifugation at 13,000 rpm for 15 min. A washing step with 1 mL of cold ethanol was applied to remove the weakly adsorbed drug on the surface. The powder dried overnight at 40 °C under reduced pressure.

2.6. Quantifying the loaded MSNs

The dried powder of loaded silica was mixed well and divided into two parts to perform the loading measurements using HPLC and TGA. For the HPLC, the IND was first extracted using three cycles of sonication for 15 min, followed by 10 min of centrifugation at 13,000 rpm. The HPLC method was used to look at the supernatant from each cycle, and the following equation (Eq. (4)) was used to determine the quantity of drug extracted in total:

$$Drug\ Loading\ (\%\, w/w) = (total\ extracted\ mass \div mass\ of\ nanoparticles)\, x\, 100 \quad (4)$$

where the total mass is the sum of the drug amount extracted from all three cycles and the mass of nanoparticles is the theoretical mass of the drug in the sample for TG analysis, the silica was loaded directly into the TGA platinum pan (the sample mass ranges from 4 to 12 mg), and its load was estimated after applying a two-step correction based on the thermogram behaviour of the as-received silica and drug, see Eq. (5).

$$Drug\ loading\ (\%\ w/w) = (\%\ mass\ loss\ x\ 100)/73.2) - 0.4 \quad (5)$$

where 73.2 is the average total mass loss (%) of IND as-received samples that can be detected in the area between 200 °C and 400 °C (the area identified in the thermograms related for the IND decomposition), and 0.4 is the mass loss (%) of the silica as-received sample.

2.7. Instrumental analysis

The HPLC analysis was adopted from a previously reported method [35]. The analysis was carried out using the Agilent system (the Agilent 1100 series, CA, USA). A C-18 column (150 × 4.6 mm, 5 μm) was used, with a mobile phase of 70:30 (v/v) methanol and water (pH = 2.7 adjusted with 1 M HCl). The flow rate was 1 mL/min in isocratic mode for 5 min. Then, a gradient flush from the isocratic to a mixture of 70 % methanol and 30 % water for 1 min before equilibrium for the next injection. The elution was obtained after 8 min at 230 nm. The injection volume was 20 μL. TGA was carried out using the TA Q500 instrument (TA, UK). The instrument was calibrated for temperature using the magnetic transition standard, which was recommended for the TA instruments. Nickel was used as a standard to measure its magnetic transition (Curie points). The instruction steps of the TA instrument were followed (see the supplementary material). The mean Curie temperature was calculated, and the measured deviation concerning the literature Curie temperature of the nickel was applied as a correction term to subsequent measurements. The analysis was performed under a nitrogen purge of 40 mL/min. The heat rate used was 10 °C/min from ambient temperature to 600 °C.

2.8. Statistical models

A simple linear regression was applied to compare the slopes of the two calibration curves obtained from the HPLC and the TGA. A two-way ANOVA conducted with Tukey's multiple comparison test was adopted to compare the results of the loading of all MSNs samples. GraphPad Prism software was used to analyse the data and plot the statistical graphs.

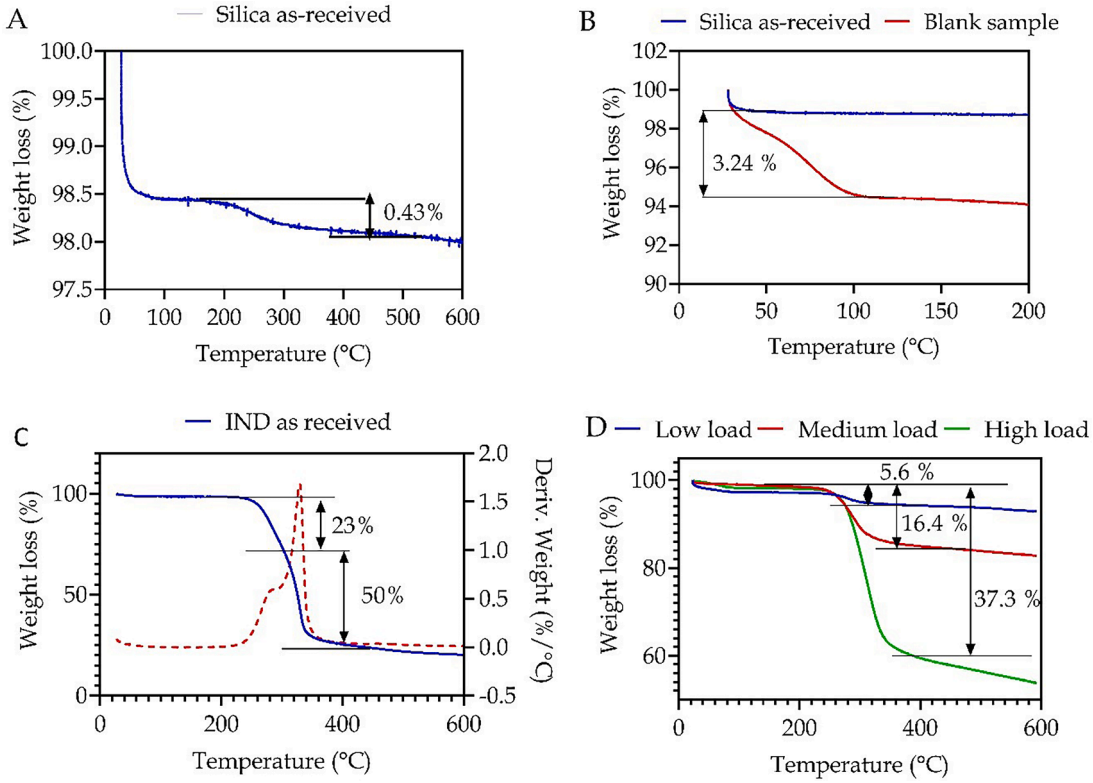

Fig. 3. Interpretation of the TGA thermogram for identifying the area of the IND decomposition. The silica as-received thermogram (A), the silica as-received vs. silica blank (silica mixed with ethanol for *24 h*) thermograms (B), the IND as-received thermogram and its corresponding 1st derivatives (C), and the different drug-loaded MSNs thermograms (D). The thermograms are representative of the repeats, and the mass losses are the average of at least three thermograms, determined using the TA analysis software.

3. Results

3.1. Morphology and surface analysis of the MSNs

MCM-41 was found to be spherically shaped (Fig. 1A), and SBA-15 was found to be rod-like (Fig. 1B). MCM-41 revealed a uniform normal distribution with an average size of 49.5 ± 11.8 nm. The rod-like shape of the SBA-15 was measured as a length and width diameter size (Fig. 1B). It also showed a uniformly distributed average length of 963.4 ± 195.1 nm and a width of 274.2 ± 47.8 nm.

The data from the BET/BJH analysis revealed a specific surface area (S_{BET}) of 1040.4 m^2/g for the MCM-41, with a pore volume (V_t) and pore size (W_{BJH}) of 0.77 cm^3/g and 1.2 nm (Fig. 2B), respectively. A smaller surface area and larger pore size and volume were obtained with the SBA-15. The specific surface area (S_{BET}) was 890.5 m^2/g, and the pore volume (V_t) and pore size were 2.0 cm^3/g and 3.8 nm (Fig. 2D), respectively. In the isotherm plots, the SBA-15 (Fig. 2C and D) showed isotherms which belonged to the IV category, indicating the presence of mesoporous structures [36]. Furthermore, hysteresis loops showed that SBA-15 belonged to the H1 type, indicating a relatively high pore size uniformity. The overall trends in the isotherms for MCM-41 indicated that it has a microporous structure [37], and the lack of a hysteresis loop can be explained by reversible nature of the nitrogen sorption into the smaller pores (Fig. 2A and B).

3.2. Interpretation of the TGA thermograms

The pre-analysis cleanliness check of the TGA revealed a small drift in the system of less than 40 μg (Fig. 3A). It was noticeable that silica was very stable at high temperatures, up to 600 °C. Nonetheless, a small step around 200 °C was recorded in the thermogram. The average mass loss (%) of this transition was 0.4 ± 0.1 %. The IND as-received samples

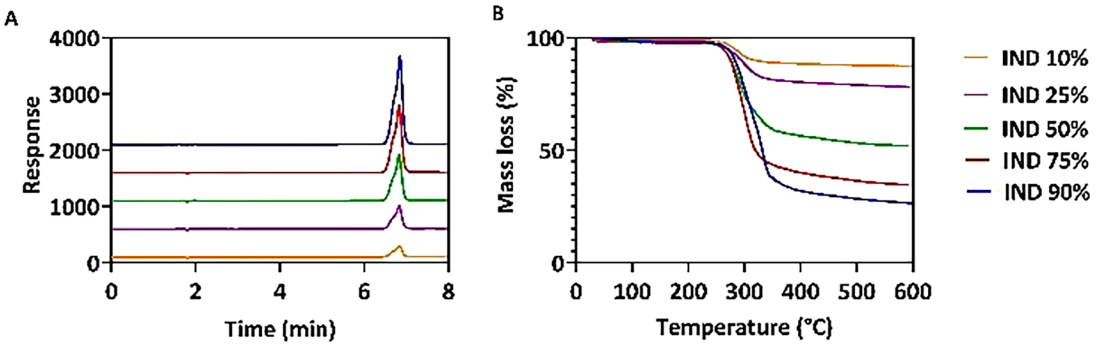

Fig. 4. The HPLC chromatograms of the difference weight ratios of the IND physically mixed with the commercial silica, MCM-41 (A), and the corresponding thermograms for the same patch obtained by the TG analysis (B).

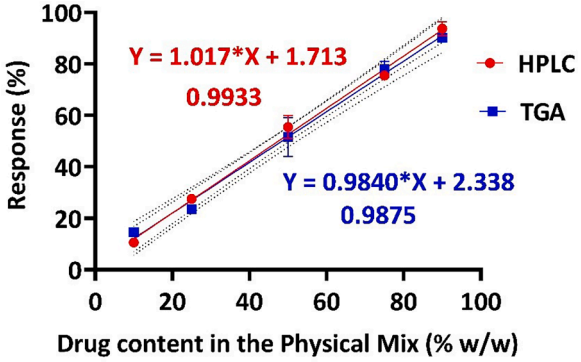

Fig. 5. A simple linear regression analysis is used to compare the slopes from both techniques' calibration curves. The analysis was performed using the GraphPad Prism software. ($n = 3 \pm$ SD). The analysis concluded that the differences between the slopes and elevations are not significant. Also, differences in elevation are insignificant. The pooled slope equals 1.00, and the pooled intercept equals 2.03. The Y axis was not normalised, which represent the drug content measurements obtained from the both the HPLC and TG analysis.

revealed a two-step transition due to drug decomposition with a total average mass loss of 73.2 ± 2.1 %, an initial mass loss of 23.0 % at 294 °C followed by 50.0 % at 318 °C (Fig. 3C). The two steps are likely to originate from separate parts and/or different functional groups of the drug molecule undergoing different degradation pathways. According to previous report [38], the drug molecule degradation can be assigned to two fragments using the negative ion mode of the mass spectrometry: $C_9H_{10}N^-$ (Exact mass = 132,08), and $C_7H_6ClN_2O_3S^-$ (Exact mass = 232, 98) [39]. The blank samples also revealed an average mass loss of 3.2 ± 0.2 % before 100 °C due to the evaporation of ethanol; after 100 °C, the thermograms were very similar to the as-received silica (Fig. 3B). It is worth noting that the blank sample is the procedural blank (the MCM-41 soaked with ethanol). The IND loaded into the MSNs showed different values of mass losses that were proportional to the initial amount of the drug used in the loading solutions (Fig. 3D). The average mass losses obtained were 5.6 ± 0.3, 16.4 ± 0.6, and 37.3 ± 0.5 % for the low, medium, and high loads of silica, respectively.

3.3. The physical mixture studies and their related calibration curves

The results from the uniformity content study performed for the physical mixture with the lowest drug content showed an acceptance value of 4.5, which is within the allowed acceptance value of the dosage form uniformity units according to the U.S. Pharmacopeia (the maximum allowed acceptance value is 15 when $n = 10$) [34]. Then, the calibration curves were plotted for the results obtained from the TG and HPLC analyses (Figs. 4 and 5). With both calibration curves, a linear relationship between the observed and expected drug content in the physical mixtures was recorded. The r^2 value was 0.9936 with a slope of 0.9816 obtained from TGA, compared to 0.9933 with a slope of 0.02942 with HPLC.

To compare the two slopes, a simple linear regression analysis was conducted, which concluded that there was no significant difference between the slopes of the two calibration curves [40]. The limit of detection (LOD) calculated for the TGA was 0.77 % (w/w), compared to 0.06 % (w/w) with the HPLC.

3.4. Adsorption method of drug loading into MSNs

The TGA thermograms of the loaded silica showed a drug loading of 2.2 ± 0.2 % (w/w) for the MCM-41 type of silica, compared to 5.1 ± 0.1 % (w/w) with SBA-15. The HPLC analysis of the same batch also revealed a drug loading of 3.6 ± 0.6 % (w/w) and 5.3 ± 0.9 % (w/w) for MCM-41 and SBA-15, respectively. To compare the drug loading results

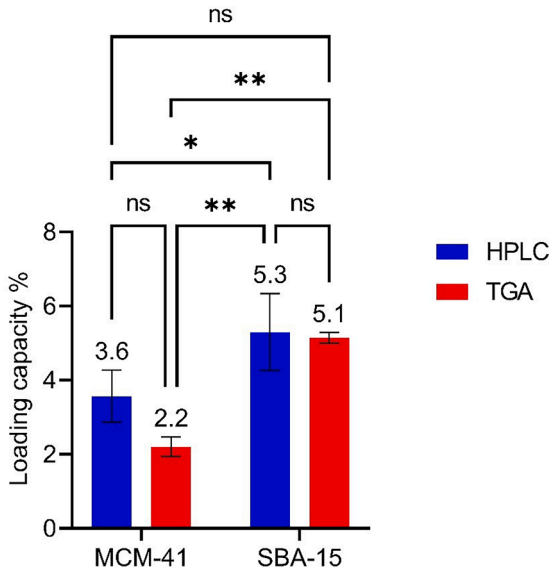

Fig. 6. The average drug loading of MCM-41 and SBA-15 was measured using HPLC and TG analysis. Tukey's multiple comparison test was adopted to compare all applied factors. The analysis concluded that there was an insignificant difference between the TGA and HPLC measurements of drug loading when applying the two types of silica. Also, it revealed a significant improvement in the drug loading of the SBA-15 obtained with both HPLC and TGA, compared to the MCM-41. ($n = 3 \pm$ SD). *= statistically significant ($p < 0.05$), and ns= statistically insignificant ($p > 0.05$).

Table 1
The BET/BJH analysis of the MCM-41 and SBA-15 before and after loading with the IND.

Sample	S_{BET} (m^2/g)*	V_t (cm^3/g)**	W_{BJH} (nm)***	Drug loading HPLC (% w/w)	Drug loading TGA (% w/w)
MCM-41	1040.4	0.8	1.4	–	–
IND-MCM-41	890.3	0.7	1.3	3.6 ± 0.6	2.2 ± 0.2
SBA-15	890.5	2.0	4.9	–	–
IND-SBA-15	693.9	1.7	5.0	5.3 ± 0.9	4.1 ± 0.1

*Surface area, **pore volume, ***pore size.

obtained from the two methods of measurement, a multiple comparison study based on Tukey's test was performed on all samples of MCM-41 and SBA-15 (Fig. 6). The analysis of the results concluded that there were insignificant differences ($p > 0.05$) between HPLC and TGA measurements in all samples examined.

Loading the IND inside the silica pores was supported by the BET/BJH analysis. Loaded samples of MCM-41 and SBA-15 showed a reduction in the specific surface area and pore volume compared to the as-received version, as presented in Table 1. In the literature, this approach has already been used to confirm the adsorption of drugs into silica nanoparticles [41].

4. Discussion

Using TGA to quantify the drug loaded into inorganic carriers, including silica nanoparticles, is often reported in confirmation with other spectrophotometric techniques [12,42]. The details of the TGA methodology for drug loading calculation were not sufficiently reported in many previous studies [12,43]. In some studies, the mass loss of the as-received drug compared to the loaded formulations was considered;

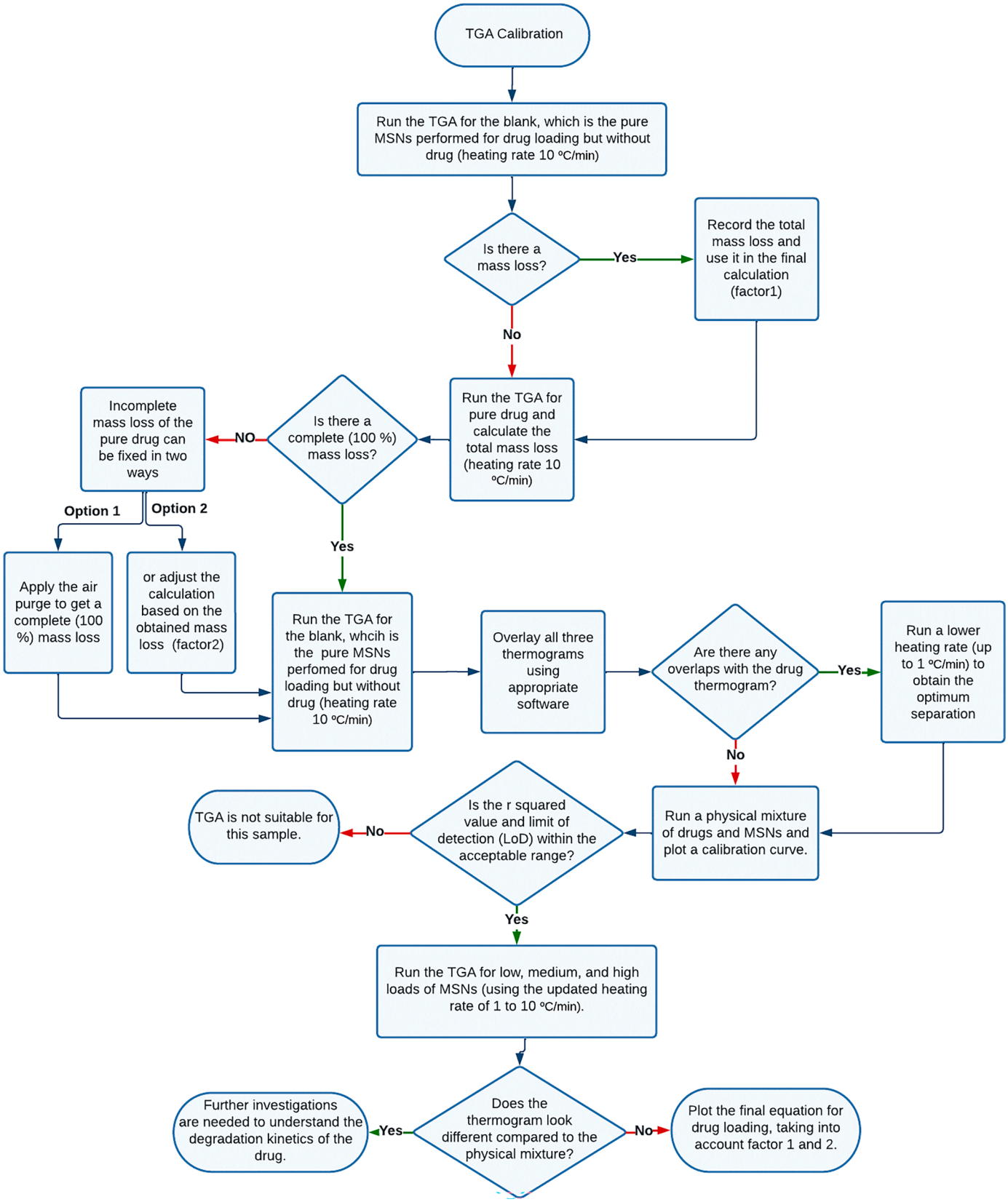

Fig. 7. A flow diagram depicting the steps proposed to optimise the TGA method for measuring drug loading in MSNs. The TGA calibration for temperature is the only requirement for performing the drug loading analysis, as weight calibration is not required in such an analysis.

however, the effects due to solvents or other possible impurities were often not studied [44]. In thermogravimetric analysis, it is crucial to know if the mass loss detected in the area of interest was only related to the compound of interest or if it was overlapping with other impurities. In the case of MSNs, there was a good chance of the presence of residual solvents due to the incomplete cross-linking of the silica matrix during the synthetic manufacturing process [45]. Therefore, all the factors that may interfere with this mass loss should be studied and considered in the calculation of drug loading to improve the selectivity of the TGA method. In this work, the effects of carrier (silica), solvent (ethanol), and degradation products of indapamide on TGA thermograms were studied. The mass loss obtained at > 200 °C with the as-received silica (0.4 ± 0.1 %) was excluded in this calculation as presented in Eq. (5). This small step transition could be attributed to the physiosorbed water and residual organic groups that were entrapped during the manufacture synthetic process [46]. Depending on the reaction rate and condition, the hydrolysis and condensation of the tetraethyl orthosilicate (TEOS), the source of silica, may not be complete, resulting in a partly cross-linked silica matrix that contains ethoxy groups in place of oxygen-bridged silica atoms. This will lead to the formation of microporous structures (~1 nm) where water, ethanol, and ammonia can be trapped [45]. It is worth mentioning that silica is synthesised by stirring TEOS with ammonium hydroxide in anhydrous ethanol. The as-received drug thermograms showed incomplete mass loss (73.2 ± 2.1 %) which may be attributed to remaining non-volatile degradation products; this was also considered for the drug loading calculation. Luckily, the mass loss obtained at ~100 °C with the silica blank sample due to the solvent residue was not interfering with the drug area of decomposition. Nonetheless, this mass loss decreased with the loaded silica samples. This suggested that the solvent competes with the drug to occupy the pores, which confirms the usefulness of this technique in this study. TGA can also be used here to assess the solvent residue and characterise MSNs for lower solvent and higher drug content. The presence of the solvent not only compromises the drug loading by occupying pore space, but some solvents can also be associated with a high risk of cytotoxicity, as reported before [47].

The physical mixture studies were investigated to evaluate the sensitivity of the TGA method for IND detection with respect to HPLC. Also, it is a way to know the thermal behaviour of IND in the presence of silica. It is possible for the drug to interact with the carrier, with a concomitant effect on the TGA thermograms. It was observed that the predicted values were in close agreement with the actual weight percent values ($r^2 = 0.9936$). It was noted that the TGA method for analysis in the presence of MSNs had an unexpected a low detection limit (LOD = 0.77 % (w/w)), implying that the highly stable inorganic material, permitted the observed ideal separation in the thermograms. Nonetheless, the detection limit for the HPLC could be overestimated as the extraction procedure for the actual loaded molecule will be much harder. That is why conducting this sensitivity evaluation using the actual drug load was more relevance. The drug loading study was performed intentionally with low amounts of the drug loaded into the silica to see if TGA could detect a small quantity of drug that was actually loaded inside the silica internal pores. The sensitivity of the TGA is well demonstrated here, as it can detect as low as 2.2 ± 0.2 % (w/w), as presented above with MCM-41. The HPLC can detect more drugs as the value of the drug loading was slightly higher, but the result showed a high variance (Fig. 6). The findings from the physical mixtures and drug loading studies, collectively, support the sensitivity of the TGA measurements. Furthermore, the TGA approach had the advantage of a single-step lower variability measurement compared to the multi-steps of HPLC.

5. Conclusions

In this work, a thorough evaluation and optimisation of the TGA approach for the drug loading measurements were carried out. The technique evaluation with respect to the standard method of HPLC analysis. The physical mixture studies demonstrated the high performance of the TGA method along with HPLC. The limits of detection (LOD) for the TGA and HPLC were 0.77 % and 0.06 %, respectively. The sensitivity of the TGA is well supported by the actual drug loading study, where the data showed that TGA can detect as low as 2.2 ± 0.2 % (w/w) of the IND loaded into MCM-41. The data showed low variability in the TGA measurements compared to HPLC. The TGA technique could be a potential alternative to HPLC to do early formulation and characterisation of MSNs. To obtain the best results using TGA, the following flowchart (Fig. 7) was suggested as an optimised method of TGA based on our findings. It is crucial to exclude the effect of the solvent residue and other possible impurities in the silica sample, or even the instrumental errors that could potentially affect the measurements. This was successfully addressed by applying the steps of the instrument calibration and the following baseline thermograms: pure silica, pure drug, and silica blank.

CRediT authorship contribution statement

M. Almaghrabi: Writing – original draft, Methodology, Formal analysis, Investigation, Visualization. **A. Alqurshi:** Writing – review & editing. **S.A. Jadhav:** Writing – review & editing. **F. Mazzacuva:** Methodology, Investigation. **A. Cilibrizzi:** Writing – review & editing. **B. Raimi-Abraham:** Supervision. **P.G. Royall:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tca.2023.179616.

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