The anticancer activity of synthetic organogermanium, β- or bis-carboxyethylgermanium sesquioxide (Ge-132), has been demonstrated in several cancer cell models and human studies. Ge-132 increases pro-inflammatory responses by enhancing interferon-γ (IFN-γ), natural killer cell and T-cell activity, and is significantly less toxic than other widely used metal-based anticancer drugs such as cisplatin. In this small-scale laboratory study, we effectively assessed the physicochemical characteristics and purity of Ge-132, our main objective being to develop a novel oral anticancer formulation, using conventional tableting excipients which do not alter the chemistry of Ge-132. We determined that solid Ge-132 decomposes at 330°C; is virtually insoluble in most common organic solvents; and readily dissolves in water (saturation solubility =1.28 g/100 ml) to form germane triol (pH 3.06–3.12). 1H and 13C nuclear magnetic resonance spectroscopy confirmed the structure of our compound showing two identical proton environments at 1.55 and 2.65 ppm (triplets) and three distinct carbon environments at 178.31, 27.37 and 12.93 ppm. The mass spectrum indicated the formation of numerous complex ion fragments with masses ranging from m/z 123.1 to m/z 478.3. FT-infrared and FT-Raman spectra showed characteristic sesquioxide peaks at 900.51, 900.26 and 800.04 cm\(^{-1}\) and, most importantly, confirmed the absence of toxic, inorganic GeO\(_2\), at 850 cm\(^{-1}\). While parenteral formulations exist for many anticancer medicines, here we successfully developed uncoated tablets containing Ge-132 (5% w/w) by manual direct compression (powder particle size ≤180 μm). The tablets passed British Pharmacopoeia (BP) content uniformity testing (Ultraviolet–visible, 212 nm), and BP disintegration testing in both acidic and basic media, disintegrating between 2 min 55 s and 3 min 10 s, respectively. We prepared gastro-resistant formulations using Eudragit*; however, these failed content uniformity tests and had lower disintegration times (≤1 min 36 s), indicating that compatibility of polymers with Ge-132 requires further investigation. The results presented here support further larger-scale research on Ge-132 as a novel metal-based oral anticancer drug which can be conveniently administered alone or included within a chemotherapy regimen. Future formulation studies on Ge-132 could focus on compatibility assessments with nano-formulations in keeping with current advancements in metal-based anticancer therapies.

**Key words:** Ge-132, spectral analysis, anticancer, oral formulation.

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**Introduction**

Despite advancements in cancer research and drug delivery, many cancers still do not have adequate targeted treatments. Modern anticancer therapies show improved clinical efficacy and specificity towards cancer cells; however, their success is limited by the development of resistance\(^1\) and numerous side effects.\(^2\)–\(^4\) Complicating their use in chemotherapy regimens. Metal-based anticancer therapy arose from the discovery of platinum (II) and platinum (IV) complexes which inhibit cell division.\(^5\)\(^,\)\(^6\) Cisplatin is one of the most successful metal-based anticancer drugs effective against a diverse range of tumour types,\(^7\) though it is associated with significant renal toxicity and induced or acquired resistance.\(^8\) The search for novel anticancer drugs continues and other metals have been investigated for their anticancer potential,\(^7\)\(^9\)–\(^20\) including germanium (Ge), a naturally occurring metalloid found in soil.\(^21\)
Ge is a constituent of many plants, including garlic and ginseng root, and may play a role in photosynthetic, self-defence and metabolic processes. The daily intake of elemental Ge from food is estimated between 367 and 3700 μg and both organic and inorganic Ge compounds have been used for many years as a dietary supplement in doses of 15 mg–1 g per day. Various synthetic organic Ge compounds have been investigated for their therapeutic efficacy in osteoporosis, malaria, hypertension, cancer and AIDS. One of the most widely researched is the sesquioxide, β- or bis-carboxyethylgermanium sesquioxide, also known as Ge-132, an organogermanium synthesized from the chloroform, HGeCl₃ (Fig. 1).

Ge-132 is thought to improve health as a potent antioxidant and enhances the immune response by inducing interferon-γ (IFN-γ) and improving natural killer (NK) cell, macrophage and cytotoxic T-cell activity. Ge-132 and novel-related synthetic derivatives have demonstrated significant anti-tumour activity in vitro in cancer cell lines and in vivo animal models of some cancers. The exact anticancer mechanism still remains relatively unclear, though induction of IFN-γ may be key. Ge-132 may also possess DNA binding specificity like cisplatin, to inhibit cancer cell proliferation.

There are reported cases of Ge-related toxicity; however, these were linked to large amounts of Ge (15–426 g) being ingested over prolonged periods, up to 36 months. Studies have narrowed acute and chronic Ge-related toxicity specifically to inorganic germanium dioxide, GeO₂ and metallic Ge which can accumulate in the liver, kidney and spleen, peripheral nerves, lungs and muscle. Since Ge-132 is also synthesized from GeO₂, it is possible that residual GeO₂ can contaminate formulations claiming to be pure organogermanium. Organogermanium compounds including Ge-132 have characteristic low toxicity and are readily excreted via the kidney with very low accumulation in major organs and tissues.

Most anticancer medicines are administered parenterally to maximize therapeutic plasma concentrations in the shortest time and to resolve bioavailability issues with other formulations. As such, platinum drugs which are aquated and activated within the cell cytoplasm require sufficient protection to bypass healthy cells and target cancer cells. Although there are several novel formulations of successful platinum-based drugs, there are limited formulation studies of other metal-based anticancer drugs (including organogermanium compounds) into viable drug delivery forms as most organometalic chemistry does not incorporate formulation science.

Parenteral drug administration is limited to hospitals or specialized treatment centres where patients rely on trained professionals using complex equipment. Additionally, parenteral formulations require sterile manufacturing conditions which increase manufacturing costs. Oral administration is still the desired route of administration for most drugs based on ease, convenience, pain avoidance and versatility of formulations, factors contributing to higher patient compliance. Thus, there is continuing need to develop effective anticancer drugs which are activated at the desired points during gastric passage.

Developing novel oral formulations, including metal-based drugs, requires proper characterization of the active pharmaceutical ingredient (API) to determine factors such as structure, purity, solubility, effect of pH and excipient compatibility. Synthetic co-polymers such as Eudragit® are commonly used tablet coatings which release APIs in a pH-dependent manner in a variety of drug-release profiles including gastric- and intestinal-dependent solubility.

In this regard, this research was designed to determine the physicochemical characteristics of Ge-132 and use various methods of spectroscopic analysis to determine the purity of Ge-132 prior to formulation. The main objective of this small-scale laboratory study was to develop simple tablets containing known amounts of pure Ge-132 as the API, using various conventional tabletting excipients. The study was also designed with the intention of developing gastro-resistant formulations which can withstand the low stomach pH to prevent or reduce gastric acid degradation, and dissolve in the higher alkaline pH of the lower gastrointestinal tract (GIT) for distribution to the target site. More importantly, as these tablets contain known amounts of API, they can be easily administered within a convenient and simple oral dosing chemotherapy regimen, incorporating the advantages of oral administration.

Materials and Methods

Materials

Carboxyethylgermanium sesquioxide (Ge-132, 99%) was obtained from Gelest, Inc. (Morrisville, PA, USA). Hydroxypropylmethylcellulose (HPMC) was obtained from The Dow Chemical Company (Midland, MI, USA). Eudragit® S-100 (ES100) and Eudragit® L-100 (EL100) were obtained from Rohm GmbH & Co. KG, Pharma

Figure 1. Step-wise synthesis of germanium sesquioxide, Ge-132, from germanium trichloride (germanium chloroform), HGeCl₃. Adapted from Chang et al.30
Polymers (Darmstadt, Germany). Microcrystalline cellulose (MCC), lactose, deionized water, deuterium water (D2O) and food colourings (red, green, blue and yellow) were generously provided by The University of Reading, School of Pharmacy (Reading, Berkshire, UK). Polyplasdone® XL-10 (Crospovidone) was obtained from ISP (Switzerland) AG. Magnesium (Mg) stearate, starch, talc and glycerol were of analytical grade and obtained from Fischer Scientific (Loughborough, Leicestershire, UK).

**Methods of analysis of Ge-132**

**Solubility testing**

5 to 10 mg samples of Ge-132 were dissolved in 25 ml of various laboratory solvents (added in 5 ml increments) with heating in an ultrasonicated bath for 5–30 min. The solvents were deionized water, 5% NaCl, 5% H2SO4, 5% HCl, dimethyl sulfoxide (DMSO), methanol, ethanol, diethyl ether and chloroform. Due to time constraints and reagent availability, testing of Ge-132 solubility in simulated intestinal fluid such as phosphate buffer (pH 6.8) was not possible at the time of this research. To determine the maximum solubility of Ge-132, 10 mg increments of the compound were added to 100 g of hot water (95°C) with stirring until no further dissolution was possible.

**pH testing**

A 1 mg/ml stock solution of pure Ge-132 in hot water (95°C) was prepared and the pH of the solution measured over 30 min (min) using a SevenEasy Mettler Toledo pH Meter.

**Melting point determination**

The melting points of five samples of pure Ge-132 were measured using a Stuart SMP10 Melting Point Apparatus.

**13C and 1H nuclear magnetic resonance spectroscopy**

13C and proton (1H) nuclear magnetic resonance (NMR) spectra were recorded at 25.1°C (298.1 K) in a Bruker Avance III 500 MHz spectrometer using D2O as a solvent. Approximately 1 mg of pure Ge-132 was dissolved in ~5 ml of solvent with gentle heating and analysed to determine the structure of the compound.

**Mass spectrometry**

A 2 mg sample was dissolved in ~2 ml of deionized water and analysed by direct injection in a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer over 60 min to determine the relative abundance of Ge-132 isotopes in the sample.

**FT-infrared and FT-Raman spectroscopy**

An FT-infrared (FT-IR) spectrum of a solid sample of Ge-132 was recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer and analysed within the mid-IR region, from 4000 to 530 cm⁻¹. A second solid sample was analysed in an FX Raman Nicolet 9600 NXR spectrometer (4000 to 100 cm⁻¹) for comparison. Finally, a Raman analysis of a saturated solution of the compound in deionized water was performed for further comparison.

**Ultraviolet–visible light spectroscopy**

Ultraviolet–visible (UV–Vis) spectroscopy was recorded on a Perkin Elmer Lambda 25 UV–Vis Spectrometer. Stock solutions of Ge-132 (1 mg/ml) were prepared in deionized water and spectra recorded from 600 to 190 nm in a quartz cuvette to determine a suitable wavelength for absorption. Serial dilutions were carried out using the stock solutions (0.5, 0.25, 0.20, 0.125, 0.0625 and 0.05 mg/ml) and spectra recorded at 212 nm using deionized water as the reference (blank) to generate a standard calibration curve of absorbance. Finally, a time-dependent scan of a 1 mg/ml sample over 15 h was performed to observe any changes in absorbance over time.

**Preparation of uncoated Ge-132 tablets**

Tableting procedures in this research were limited due to the scale of the study. Uncoated biconvex tablets containing Ge-132 as the API were prepared by direct manual compression of dry powder blends containing the API and conventional pharmaceutical tableting excipients. An initial test formulation (Formulation 1) was prepared primarily to determine a suitable bulk mass for dry powders using lactose (filler) and MCC (compression aid and dry binder). This formulation was also used to determine compressibility and compression parameters for the dry powder as well as an optimal tablet weight, as the excipients comprised majority of the dry powder.

With the exception of Formulation 1, Ge-132 content was maintained conservatively at 5% (250 mg) for all formulations in 5 g bulk powder batches (Table 1). Lactose content was kept at 40–50% to increase the bulk powder volume. MCC was included at 25–40% to improve compression and adhesive properties of the powders. Mg stearate (lubricant) was maintained at 1% to promote powder flow. Tablet disintegrants (starch and crospovidone) were maintained at 10%. HPMC was added as an additional binder and coating between 4% and 10% of the dry weight.

The powders were ground in a mortar and sieved in a Fritsch Vibratory Sieve Shaker to achieve a particle size of ≤180 μm. Bulk powders were mixed in a rotary Turbul Type 2C Mixing System for 2.5 min then compressed in a RIVA Minipress MII bench-top eccentric single-punch tablet press, maintaining the die cavity at a depth of 7 mm.

**Preparation of Eudragit® dispersions and solutions**

ES100 and EL100 aqueous dispersions and organic solutions were prepared using slight modifications of previous research.25–35 (Table 2).
Preparation of ES100 and EL100 aqueous dispersions
Granules of each polymer were dispersed in deionized water at room temperature with continuous high-speed stirring for 60 min using an IKA RCT Basic heated magnetic stirrer. To partially neutralize the carboxylic groups of the polymers, 1 M ammonium hydroxide was added. A separate mixture of talc (glidant) and glycerol (plasticizer) was prepared and stirred for 30 min. The glycerol–talc mixture was gradually added to the polymer dispersion with constant stirring. Finally, two drops of food colourings (blue and green) were added and mixing continued for a further 60 min.

Preparation of ES100 and EL100 organic solutions
Granules of each polymer were gradually dispersed into 96% ethanol with continuous high-speed stirring for at least 30 min at room temperature. A glycerol–talc mixture and food colourings (red and yellow) were added to the polymer solution and stirring continued for a further 60 min.

Incorporation of aqueous ES100 and EL100 into dry powders
Due to the small volumes of bulk powder used in this research, we attempted manual film coating of the tablet surfaces; however, limitations in the adjustment of droplet size prevented uniform coating. As an alternative, bulk dry powders of Formulation 4 were prepared with ES100 and EL100 aqueous dispersions incorporated into the mixture at 20–22% of the dry powder weight, prior to direct compression (Formulations 5–9) (Table 3). The polymer mixtures were added dropwise with continuous high-speed mixing using a magnetic stirring bar to break up agglomerates. Mixing was continued for a total of 20 min until a relatively uniform powder (by sight) was formed. The mixture was left to dry at room temperature for 60 min, ground and sieved to obtain particles of ≤75 μm to enhance powder flow and compressibility. The final powder was reweighed, mixed in a rotary mixer and compressed into tablets with the die cavity adjusted to 8.5 mm to accommodate more dry powder.

Quality testing of tablets
All formulated tablet batches were subjected to various standard quality tests of the British Pharmacopoeia (BP) 2010, including consistency of formulation and disintegration testing. Uniformity of weight (Mass)
Twenty tablets from each batch were individually weighed on a Mettler AT261 Delta Range scale. Average weights

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**Table 1. Preparation of uncoated Ge-132 tablets using conventional excipients (all weights in grams)**

|                      | Formulation 1 (test) | Formulation 2 | Formulation 3 | Formulation 4 |
|----------------------|----------------------|---------------|---------------|---------------|
| API                  |                      |               |               |               |
| Ge-132               | 0.5                  | 0.25          | 0.25          | 0.25          |
| Filler               |                      |               |               |               |
| Lactose              | 3.75                 | 2.5           | 2.5           | 2             |
| Compression aid/dry binder | 1.875     | 1.25          | 1.25          | 2             |
| Lubricant            |                      |               |               |               |
| Mg stearate          | 0.06                 | 0.05          | 0.05          | 0.05          |
| Binder/disintegrant  |                      |               |               |               |
| Crospovidone         | –                    | –             | 0.5           | 0.5           |
| Starch               | –                    | 0.5           | –             | –             |
| Dry coating          |                      |               |               |               |
| Hydroxypropylmethyl cellulose (HPMC) | –          | 0.45          | 0.5           | 0.2           |
| Total dry powder weight | 6.185            | 5.0           | 5.05          | 5.0           |
| % API content        | 8%                   | 5%            | 4.95%         | 5%            |

**Table 2. Preparation of Eudragit® S100 and L100 aqueous and organic formulations (all weights in grams)**

|                      | Eudragit® S100 | Eudragit® L100 |
|----------------------|----------------|----------------|
| Aqueous Dispersions  |                |                |
| Mass of polymer      | 10             | 10             |
| Water                | 50             | 50             |
| NH₄OH (1 M)          | 5              | 5              |
| Talc                 | 0.5            | 0.5            |
| Glycerol             | 5              | 5              |
| Organic solutions    |                |                |
| Mass of polymer      | 5              | 5              |
| Ethanol (95%)        | 75             | 75             |
| Talc                 | 0.25           | 0.25           |
| Glycerol             | 0.5            | 0.5            |

Preparation of ES100 and EL100 aqueous dispersions
Granules of each polymer were dispersed in deionized water at room temperature with continuous high-speed stirring for 60 min using an IKA RCT Basic heated magnetic stirrer. To partially neutralize the carboxylic groups of the polymers, 1 M ammonium hydroxide was added. A separate mixture of talc (glidant) and glycerol (plasticizer) was prepared and stirred for 30 min. The glycerol–talc mixture was gradually added to the polymer dispersion with constant stirring. Finally, two drops of food colourings (blue and green) were added and mixing continued for a further 60 min.

Preparation of ES100 and EL100 organic solutions
Granules of each polymer were gradually dispersed into 96% ethanol with continuous high-speed stirring for at least 30 min at room temperature. A glycerol–talc mixture and food colourings (red and yellow) were added to the polymer solution and stirring continued for a further 60 min.
and standard deviation for each batch were calculated and analysed using BP guidelines to assess batch quality.

**Disintegration tests**

Disintegration testing was performed using a pre-assembled 6-well basket rack in a Copley Erweka ZT42 Disintegration Apparatus maintained at 37°C. For each batch, six randomly selected tablets were placed into the basket-rack and covered with a 20 mm plastic disc. The basket was lowered into 700 ml of immersion fluid and the apparatus operated for 15 min. The immersion fluids were 0.1 M HCl (pH 1.36) and deionized water (pH 7.4). The disintegration time was recorded as the time for the last of the six tablets to fully disintegrate. Disintegration tests were performed twice for each formulation in each of the immersion fluids.

**Uniformity of content**

Uniformity of content testing was carried out on Formulations 2–9 according to BP guidelines. Ten tablets were individually weighed, crushed and dissolved in 1000 ml of hot deionized water, then filtered to remove undissolved excipients. Two millilitres of samples were analysed by UV–Vis (212 nm) and the absorbances used to calculate the content of Ge-132 in the tablets (against the standard calibration curve). The acceptance values (AVs) for deviations were calculated according to BP guidelines.

**Results**

**Analysis of Ge-132**

**Solubility, pH and melting point**

Ge-132 fully dissolved in water forming an acidic solution of pH 3.06–3.12, and fully dissolved in common aqueous laboratory solvents including 5% NaCl, 5% H2SO4 and 5% HCl. The compound was insoluble in common organic laboratory solvents (methanol, ethanol, diethyl ether and chloroform) and only partially soluble in DMSO with heating.

The melting point of the compound proved too high to measure using the laboratory apparatus available (temperature limit of 350°C). At 330°C, Ge-132 decomposed to a brown solid with no visible droplets forming to indicate melting.

**1H and 13C NMR spectra of Ge-132**

The 1H NMR (D2O) spectrum showed two identical proton environments at 1.55 and 2.65 ppm, and the 13C NMR (D2O) spectrum showed three distinct carbon environments at 178, 27 and 12.5 ppm. The peaks in both spectra were consistent with the proposed molecular structure and monomeric formula of Ge-132.

**Mass spectrum of Ge-132**

Hydrolysis of the compound resulted in numerous fragments of varying mass presented in the full mass spectrum of Ge-132 (Fig. 2). The complex spectrum shows the relative abundances of distinct Ge-containing isotope clusters with splitting patterns very similar to those previously reported. Additionally, these clusters occur in suggested overlapping regions as indicated. These clusters indicate either incomplete fragmentation of the solid structure or formation of new linked Ge-132 units, possibly cyclic and linear.

**FT-IR and FT-Raman spectroscopy of Ge-132**

The FT-IR spectrum of the solid compound shows characteristic carboxylic O–H bond and C=O bond vibrational peaks at 3300.00–2700.00 and 1687.55 cm⁻¹, respectively (Fig. 3). The peaks at 1410.00 and 1236.65 cm⁻¹ indicate asymmetrical and symmetrical C–H bond vibrations of the alkyl chains. Most notable in this mid-IR spectrum are the prominent characteristic sesquioxide peaks at 900.51 and 800.04 cm⁻¹, consistent with previous studies of pure Ge-132 and its derivatives confirming the –O–Ge–O–Ge-type network.

The comparative FT-Raman spectrum of solid Ge-132 (Fig. 4, top) shows more distinct carboxylic O–H bond

| Table 3. Preparation of Ge-132 5% tablets with incorporated Eudragit® S100 and Eudragit® L100 (all weights in grams) |
|------------------------------------------------------|
| Formulation | 5 | 6 | 7 | 8 | 9 |
| Ge-132 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lactose | 2 | 2 | 2 | 2 | 2 |
| MCC | 2 | 2 | 2 | 2 | 2 |
| Mg stearate | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Crospovidone | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| HPMC | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Eudragit® S100 | 1 | – | 0.5 | 0.8 | 0.35 |
| Eudragit® L100 | – | 1 | 0.5 | 0.4 | 0.7 |
| Final dry powder weight | 4.10 | 4.08 | 4.14 | 4.23 | 4.05 |
vibrations at 2932.90 cm\(^{-1}\) as well as the characteristic sesquioxide peak at 900.26 cm\(^{-1}\). New peaks were observed at 688.02 and 445.57 cm\(^{-1}\), the former peak indicating Ge–C bond vibrations.\(^6\)\(^2\) The Raman spectrum of aqueous Ge-132 in Fig. 4 (bottom) shows the vibrational frequencies of the hydrolysis products of Ge-132 with broader peaks observed at 3361.96, 1643.06 and 1437.44 cm\(^{-1}\).
UV–Vis spectroscopy of Ge-132

Between 215 and 210 nm, the serial dilutions we prepared showed consistent absorption within these wavelengths as well as relatively minimal variation in the gradient between the serial dilutions. For this reason, 212 nm was chosen as a suitable wavelength to study Ge-132 UV absorbance and a resulting calibration curve produced (Fig. 5). Over 15 h, a 1 mg/ml solution of Ge-132 had constant absorbance ($A=0.287$) at 212 nm.

Analysis of tablet formulations

Tablets of Formulations 1–5 measured 6 mm in diameter with a thickness of 2 mm, whereas Formulations 6–9 were slightly thicker at 4–5 mm with a similar surface diameter.

Formulation 4 containing a 1:1 lactose:MCC mixture yielded a powder with better compressibility than Formulations 1–3 which had a 2:1 (lactose:MCC) mixture. This formulation was therefore chosen for further development in Formulations 5–9.

Uniformity of weight (Mass) test

Tablets of Formulations 2–9 passed BP test of Uniformity of Weight (Mass) with no sample deviating above or below the mean respective batch weight by >7.5%.56 Tablets in Formulations 2–4 had mean weights of 116.45 ± 3.06, 110.33 ± 4.06 and 109.38 ± 3.75 mg, respectively. As expected, tablets in Formulations 5–9 were heavier due to incorporation of aqueous Eudragit®. Mean weights were 123.40 ± 3.15 mg (Formulation 5), 148.11 ± 2.13 mg (Formulation 6), 133.26 ± 1.79 mg (Formulation 7), 131.75 ± 3.49 mg (Formulation 8) and 134.01 ± 2.89 mg (Formulation 9).

Tablet disintegration tests

Disintegration times for all formulations were determined in both acidic and alkaline media. Tablets of Formulation 1 fully disintegrated within 13 s in both solutions which was expected, considering the powder did not contain significant amounts of binder. Formulations 2–4 had longer disintegration times, between 2 min 55 s in acidic pH and 3 min 10 s in alkaline pH. Formulations 5–9 with Eudragit® incorporated into the bulk powder had lower disintegration times (1 min 13 s to 1 min 36 s), though this was due to the smaller particle size.

Uniformity of content test

Mean absorbances at 212 nm ranged from 0.114 to 0.913. Using our calibration curve, we converted the absorbances into mean API content with the following results: Formulation 2, 4.95%; Formulation 3, 4.68%; Formulation 4, 4.80%; Formulation 5, 5.02%; Formulation 6, 5.18%; Formulation 7, 2.45%; Formulation 8, 3.73%; and Formulation 9, 0.65%. The mean % Ge-132 content for each formulation was then analysed using the BP formula for calculating AV:

$$|M - \bar{X}| + ks$$

where $M$ is the reference value from the mean API content as a percentage of the label claim; $X$ the mean content; $k$ the acceptability constant or tolerance interval (2.4 for 10 tablets); and, $s$ the standard deviation.

An AV of ≤15 (termed L1) denotes a successful formulation.

The AVs were calculated as 0.01, 4.87, 2.51, 0.00, 2.13, 49.71, 24.49 and 86.07 for Formulations 2–9, respectively.

Discussion

Solubility, pH and melting (decomposition) point

Ge-132, a white, crystalline powder, exists as a complex, infinite crystal network of O–Ge–O bonds with the basic monomeric formula $O_3(GeCH_2CH_2COOH)_2$.57 (Fig. 6A). Ge-132 fully dissolves in water and equilibrates to the trihydroxyl, germane triol, $(OH)_3GeCH_2CH_2COOH$ (Fig. 6B), forming an acidic solution (pH 3.06–3.12). Across all tested solvents, water provided the best dissolution medium with the saturation solubility of Ge-132 determined to be ~1.28 g per 100 g of water. We also observed that even after cooling and over a period of 5 days, the compound remained in solution and did not precipitate out. Stability testing of the compound in solution over longer periods of time is an important aspect of future research which will provide further data on the viability of Ge-132 as a novel anticancer drug.

The melting (decomposition) temperature we measured at 330°C is consistent with previously reported literature and also expected from the known melting temperature of elemental Ge of 937.4°C. The high melting (decomposition) point is attributable to the crystal structure resulting from complex polymerization of the monomeric unit.
NMR spectroscopy

$^1$H and $^{13}$C NMR were performed to confirm the structure of Ge-132. D$_2$O was chosen as the analysis solvent due to the favourable solubility in water. In the $^1$H NMR spectrum, the triplet peaks at 1.55 and 2.65 ppm indicate proton pairs in the $-\text{Ge}–\text{C}_2\text{H}_2–\text{CH}_2–$ and $-\text{CH}_2–\text{C}_2\text{H}_2–\text{COOH}–$ regions, respectively. These proton environments are distinguishable by the proximity of the latter protons to the electronegative carboxyl oxygens, which shifts them further downfield on the chemical shift ($\delta$) scale. The $\delta$ values are consistent with previously reported values of 1.61 and 2.69 ppm.\textsuperscript{58}

The peaks presented in the $^{13}$C NMR spectrum were also consistent with the expected molecular structure and structural data for pure Ge-132 previously reported.\textsuperscript{57,58} These peaks confirm the presence of carbons as follows: 178 ppm, Ge–CH$_2$CH$_2$–; 27 ppm, Ge–CH$_2$–; 12.5 ppm, Ge–CH$_2$–COOH. These $\delta$ values are comparable to those previously as 181.30, 28.43 and 15.68 ppm.\textsuperscript{58}

MS spectrometry

Elemental Ge exists in five known stable isotopes—$^{70}$Ge (20.5%), $^{72}$Ge (27.4%), $^{73}$Ge (7.8%), $^{74}$Ge (36.5%) and $^{76}$Ge (7.8%).\textsuperscript{61} This polyisotopic nature produces a complex MS spectrum with significant overlapping regions consisting of different ions. Furthermore, these ions are polymeric (monomeric, dimeric, trimeric, etc.) depending on the number of Ge atoms, and vary in mass depending on the constituent Ge isotopes within each ion.

The smallest linear units are possibly the monomeric ions (OH)$_3$Ge+, (OH)$_3$GeCH$_2$CH$_2$ and (OH)$_3$GeCH$_2$CH$_2$COOH$^+$ with calculated masses of $m/z$ 125, 153 and 199, respectively. These ions are present in our MS spectrum at $m/z$ 123.1, 151.1 and 193. Similarly, the basic cyclic units can be any of several different forms (Fig. 7A–E) ranging in mass from $m/z$ 181 to 478.3. Another complication of interpreting the MS of Ge-132 is fragmentation from addition or loss of neutral species—such as H$_2$O, CO$_2$, CH$_2$¼CH$_2$ and GeO—can change the shape and isomeric nature of the ion.\textsuperscript{59} For example, the prominent peak at $m/z$ 213.1 may be the linear dimeric fragment (OH)$_3$GeOGe$^+$, or the cyclic dimeric fragment in Fig. 7C resulting from the loss of CO$_2$ and CH$_2$¼CH$_2$. Similarly, the peak at $m/z$ 193.0 could be the linear monomeric ion (OH)$_3$GeCH$_2$CH$_2$COOH$^+$, or possibly the linear dimeric ion HOGeOGeH$_2$. The spectrum is further complicated by the polyisotopic nature of Ge where each isotope produces its own characteristic splitting pattern. This means that any combination of isotopes can be present in a fragment causing overlapping peaks which may not be visible in the spectrum or identifiable in Fig. 2.

For the purposes of interpreting our spectrum, all masses were calculated using the most abundant isotope, $^{74}$Ge, which accounts for the differences in $m/z$ values between

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**Figure 6.** (A) Structural representation of the basic monomeric unit of solid Ge-132, where R = CH$_2$CH$_2$COOH, and $n$ denotes the degree of polymerization (adapted from Sawai et al.\textsuperscript{58}). (B) Formation of germane triol (trihydroxygermane) from hydrolysis of solid Ge-132.

**Figure 7.** Proposed structures of some cyclic ions identified from peaks in the MS analysis of pure Ge-132 in aqueous solution, showing monomeric (A), dimeric (B and C) and trimeric (D and E) ion fragments of Ge-132 ($^{74}$Ge) with predicted and actual $m/z$ values. The loss of neutral species alters the shape of the ions, for example, structure C results from the loss of neutral CO$_2$ and CH$_2$¼CH$_2$ from structure B (adapted from Wie et al.\textsuperscript{59}).
our calculated and measured masses. Further detailed MS analysis beyond the scope of this research is required to accurately determine the exact isotopic composition of the compound and conclusively ascertain the identity of the fragments. The spectrum however remains significant as it further confirms the purity and solubility products of our API and, most importantly, the absence of fragments of inorganic GeO₂.

**IR spectroscopy**

For additional confirmation of the purity and constituent bonds in Ge-132, both FT-IR spectroscopy and FT-Raman spectroscopy were performed. Inorganic GeO₂ absorbs strongest at 850 cm⁻¹ and produces a distinct characteristic peak in both FT-IR and Raman spectra. For identification of functional groups which may not be visible in the FT-IR spectrum, FT-Raman spectroscopy was performed to detect weaker vibrational frequencies in the far-IR region below 600 cm⁻¹. Most notable is the peak observed at 445.57 cm⁻¹; however, without further analysis, the identity of this peak remains unknown and to date, there are no reported studies identifying this peak. Most important for this research was conclusive confirmation of the absence of toxic, inorganic GeO₂ (at 850 cm⁻¹).

The broader peaks at 3361.96, 1643.06 and 1437.44 cm⁻¹ in the Raman spectrum of aqueous Ge-132 denote bond vibrations from –COOH and alkyl chain hydrolysis consistent with the MS fragments presented above. Of particular interest is the absence of the sesquioxide peaks at 800 and 900 cm⁻¹ previously seen in the FT-IR and Raman spectra of solid Ge-132, indicating hydrolysis of the crystal network and formation of newer –O–Ge–O– links as seen in our MS. Finally, the peak between 890.86 and 708.49 cm⁻¹ in Fig. 4 (bottom) has been identified as characteristic Ge–H bond vibrations within the trihydroxylgermane, H₃Ge–(OH)₃. The absence of most peaks between 1400.00 and 601.37 cm⁻¹ is further evidence of the formation of new hydrolysis products and, as seen previously, the GeO₂ peak at 850 cm⁻¹ remains absent.

**UV–Vis spectroscopy**

Various wavelengths for UV–Vis spectroscopy (in water) have been reported for Ge-132, from 190 to 210 nm. Because these wavelengths occur at the lower detection limit of our spectrometer (190 nm), a series of scans from 220 to 190 nm were performed on dilute Ge-132 solutions. At 212 nm, the absorbance series of the serial dilutions were most linear and this was chosen as a suitable wavelength for this research. The 15 h (900 min) time-dependent UV–Vis absorbance at 212 nm showed constant absorbance (A = 0.287), indicating no further decomposition of the compound in solution and the overall stability of the hydrolysis products.

**Ge-132 tablet formulations**

Preparation of most tablet formulations involves multistep processes including dry granulation, wet granulation and fluid bed drying, prior to compression. However, due to the small-scale nature of this study, preparation methods were limited to direct compression. All tablets were biconvex and produced by manual rotation of a single punch press. BP tests of resistance to crushing and tablet friability were not performed at the time due to technical failure of the available equipment. The higher loss in bulk powder weight prior to compression in Formulations 5–9 can be attributed to the extra processing steps (mixing, grinding, sieving and choosing a fraction of ≤75 μm) used to incorporate Eudragit® into the bulk powder.

Two interesting observations were made: first, despite preparing Formulation 5 with the reduced particle size and increased die cavity depth, the tablets measured 6 × 2 mm, similar to Formulations 1–4. It is possible that ES100 may have altered the powder characteristics or the processing may have changed the flow and compaction properties of the powder, although the exact reason requires further investigation. Second, tablets of Formulation 6 were notably heavier (mean weight 148.11 mg) and thicker (5 mm), despite the same particle size and die cavity depth as Formulations 5, 7, 8 and 9. This may also be attributed to the constituent polymer (EL100) or the powder processing and emphasizes the need for further formulation studies. The fast disintegration times across all formulations are also attributable to the tablet manufacturing process using dry granulation and direct manual compression of the dry powders. Wet granulation with a liquid binder to sufficiently wet the dry powders would be a more effective method of producing homogenous granules of API and excipients with better compaction properties. However, the disintegration times reported here provide a baseline for further formulation studies using alternative granulation methods. Increasing the tablet dimensions by modifying the tabletting machine parameters may also improve disintegration times as demonstrated by Obeidat et al. but should be made with consideration of the purpose of any oral formulation, bearing in mind various patient factors.

There are various possibilities why Formulations 7–9 failed content uniformity testing, determined by the calculated AV. The polymer combinations within each formulation may have altered the API characteristics; alternatively, the extra mixing step and decreased particle size after polymer incorporation may have resulted in particle segregation which decreases powder quality and affects the distribution of API particles. Interestingly, a previous study using ES100–EL100 combinations as tablet coatings for the acidic drug mesalazine determined that a higher EL100:ES100 ratio produced a more favourable drug-release profile when the ratio was kept above 4:1.
Further large-scale studies using fluidized spray coating will provide useful data on the compatibility of polymer combinations with Ge-132 to formulate a novel gastro-resistant anticancer treatment. The formulation studies must also consider the reported solubility of Ge-132 in water, the primary solvent used to prepare the polymer suspensions.

**Eudragit® tablet formulations**

A range of Eudragit® co-polymers are widely used to produce various modified-release profiles determined by the relative ratio of methacrylic acid to methyl methacrylate ester within each polymer. In ES100, this ratio is 1:2 (acid:ester), making it selectively soluble at pH > 7.0 around the ileum and colon.53 The constituent ratio in EL100 is 1:1, making it soluble at a slightly lower pH (6–7) and useful for drugs targeting the jejunum.52,53 ES100 and EL100 have very similar physicochemical characteristics and for this reason were prepared in the same way.51

Dispersion of dry Eudragit® powders in water and addition of ammonia (1 M) produced a milky latex. This consistency allowed us to gradually incorporate the Eudragit® dispersions dropwise into our dry powders as an alternative to wet granulation. In contrast, ES100 and EL100 formed glossy and highly viscous solutions in ethanol which prevented dropwise addition. Moreover, use of organic coatings is steadily declining in the pharmaceutical industry due to environmental and health concerns.53 Despite variable results from Formulations 5–9, future research of gastro-resistant Ge-132 formulations should aim to maintain the particle size between 75 and 180 μm, in addition to analysing the actual effect of polymers such as Eudragit® on Ge-132.

**Conclusion and future studies**

The use of metals in medicine has significantly increased, particularly in developing novel anticancer therapies. The anti-tumour activity of Ge-132 has been reported in numerous studies warranting further development as a novel anti-cancer drug. Despite the small scale of this study, we were able to determine the purity of Ge-132 using common spectroscopic techniques which are also efficient methods of detecting the presence of toxic GeO2.

Nearly, all anticancer drugs are administered parenterally, despite the oral route being the most popular route of administration due to its ease, convenience and general patient acceptability. Ge-132, a freely water-soluble compound, is readily compatible with common tabletting excipients. Although not a highly targeted formulation, the tablets presented here provide a convenient and simple novel oral formulation for possible administration within a chemotherapy regimen, supporting our main objective. Variable results from tests of our gastro-resistant oral formulations warrant further large-scale formulation studies to assess the compatibility and suitability of polymers coatings such as Eudragit® with Ge-132, taking into account the acidic nature and solubility characteristics of Ge-132. Additionally, further research should also appropriately determine Ge-132 solubility in simulated intestinal fluid or phosphate buffer (pH 6.8) to mimic the natural passage of oral formulations through the GIT.

Finally, with advancements in nanotechnology and targeted drug delivery, future research might also focus on development of nano-formulations of Ge-132 beyond conventional gastro-resistant tablets. This research could evaluate the compatibility of Ge-132 with various nanocarriers and formulation of the compound into novel nanoparticulate systems such as Ge-132-polymer conjugates, liposomes and micelles.

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**Author biography**

S.M.O. graduated in July 2010 with an undergraduate Masters degree in Pharmacy from School of Pharmacy, University of Reading, UK. She is currently undertaking 1 year Pre-registration Pharmacist training in hospital pharmacy. Special interests include paediatric pharmacy and formulation of drugs for paediatric administration. Therapeutics areas of particular interest include cardiology, cancer and HIV. She is hoping to pursue a PhD in pharmaceutical formulation.

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