

Evaluation for collagen products for cosmetic application

YONG PENG, VERONICA GLATTAUER,
JEROME A. WERKMEISTER, and JOHN A. M. RAMSHAW
*CSIRO Molecular Science, 343 Royal Parade, Parkville,
Victoria 3052, Australia.*

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Synopsis

Collagen is an important component for cosmetic formulation, where it is an effective natural humectant with high substantivity. Commercial collagen preparations have a wide range of properties. In the present study, various techniques have been used to examine three distinct commercial collagens that illustrate the range of properties that are available. The usefulness of the various techniques for assessing collagen quality and batch-to-batch variation is discussed. The results indicate that there are several simple, cheap, and effective methods, such as gel electrophoresis, that provide excellent information on collagen quality. The appropriate selection of tests allows informed decisions on the choice of which collagen preparation to use to provide the desired functionality and shelf life of a formulation.

INTRODUCTION

Collagen has become a valuable and well used component in cosmetic formulation, where it provides significant benefits. In particular, it is an effective natural humectant (1,2) as a result of the extensive, ordered hydration network that surrounds the molecule (3), in combination with its high substantivity to the skin surface. A wide variety of collagen preparations is now available that are suitable for cosmetic formulations. These preparations vary considerably in their composition and properties. Quality testing to allow informed decisions on the choice of which collagen preparation to use is therefore important so as to ensure that the preparation provides the desired functionality. Testing should also be capable of monitoring potential batch-to-batch variations that could also affect product performance. Also, it should indicate whether the collagen is in its native, triple-helical form or is present in its denatured form, gelatine.

Native collagen is characterized by a triple-helical structure, in which three extended left-handed polyproline II-like helical chains are supercoiled into a right-handed triple helix, linked through interchain hydrogen bonds (4,5). The triple-helical conformation requires a distinctive amino acid sequence, in particular glycine as every third residue,

Address all correspondence to John A. M. Ramshaw.

to accommodate the close packing of the three chains. The extended conformation of the individual polyproline II-like chains in the triple helix is stabilized by the high level of occurrence of the amino acids proline and 4-hydroxyproline (Hyp). Hyp, which is post-translationally modified from proline, confers a greater stability than proline in the Y position (6) and is a characteristic amino acid marker of collagen. In tissue, the collagen molecules are crosslinked to form an extended network that provides the tissue with strength and durability. These crosslinks are specific and use short, non-triple-helical peptides, the telopeptides, at the ends of the triple-helical domain of each collagen molecule (7).

Bovine collagen has been a common source for cosmetic applications. However, more recently, collagens from other species of origin have also become commercially available. Collagens from alternative species may provide a benefit in that potential zoonoses, particularly transmissible spongiform encephalopathies, are less likely from sources such as chicken and fish. However, collagens from various species may differ in their properties. For example, they may have different thermal stabilities (8), which could affect formulation or the shelf life of products.

In the present study we have examined the suitability of a range of tests, using a selection of commercially available collagen samples. These samples illustrate a selection from the range of materials available and allow commentary on the information that each test provides. Some tests, those found in water analysis standards (9), for example, are readily available from many analytical laboratories, whereas other tests, which may be critical for providing discrimination between samples, may require the involvement of more specialized laboratories.

MATERIALS AND METHODS

●COLLAGEN SAMPLES

Commercial collagen samples suitable for cosmetic application were obtained from the following sources: Collasol[®] was from Croda Chemicals Ltd (Humberside, UK). CLR Collagen[®] was from Chemisches Laboratorium Dr. Kurt Richter GmbH (Berlin, Germany). AteloHelogen[®] was a gift from the manufacturer (Meddicoll, North Ryde, Australia). Soluble collagen samples were also prepared in our laboratory from fresh, minced dermis (bovine, porcine, and chicken, obtained from local abattoirs) by pepsin digestion using 1 mg/ml pepsin in 100 mM acetic acid adjusted to pH 2.5 with HCl (10). Soluble collagen from diced fish skin (Blue Grenadier (*Macruronus* sp.) and Nile perch (*Tilapia* sp.) obtained from a local supplier) was extracted using 0.1 mg/ml pepsin in 100 mM acetic acid adjusted to pH 2.5 with HCl. All collagens were purified by differential NaCl fractionation at pH 2.5 and then at pH 7.4 (11). Separation of type I and III collagens was by rapid ammonium sulfate fractionation (11).

METH●DS

Solution analysis. Analyses for ash, arsenic, and heavy metals (as lead) were performed by a certified external analytical laboratory (MGT Environmental Consulting Pty. Ltd., Oakleigh, Australia) following standard procedures (9). pH was determined using a

Radiometer PHM240 instrument (Copenhagen, Denmark). Conductivity was determined using a Radiometer CDM3 instrument.

Water regain. Collagen samples were dried by lyophilization in preweighed bottles with stoppers and then held over silica gel for more than 72 h. Samples were then stoppered and reweighed. They were then transferred and opened in an environment of 32% constant relative humidity, maintained over saturated CaCl₂ solution. After equilibration for more than 48 h, the samples were stoppered and reweighed. Water regain by the dried samples was determined, and expressed as a percentage of the dry weight.

Spectroscopy. For UV/visible spectroscopy, spectra were collected from 400 nm to 650 nm on a Shimadzu UV-265 recording spectrometer using cells of 10-mm path length on collagen solutions as supplied or after dilution to equal concentrations of 1 mg/ml with milliQ water. For IR spectroscopy, collagen samples were lyophilized and then examined using a Perkin Elmer 2000 AutoImage spectrometer with the ATR accessory. Alternatively, samples may be analyzed as KBr disks.

Electrophoresis. Collagen samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12), using 5% (w/v) running gels. Prior to analysis, collagen samples in sample buffer were neutralized, if necessary, with 2 M Tris and then heated at 100°C for 2 min. Separation of the α 1(I) and α 1(III) chains was by reduction with 2-mercaptoethanol during interrupted electrophoresis (13). Collagen samples were also examined by a non-denaturing, lactic acid buffer system, pH 3.1 (14), using 4% running gels. Estimation of the isoelectric point, pI, was by examination of the direction of electrophoretic mobility in gel electrophoresis in 5% running gels in a Tris/Borate pH 8.9 system (15).

Amino acid analysis. Lyophilized collagen samples were hydrolyzed *in vacuo* by constant boiling (5.8 M) of HCl containing 0.1% phenol in a Waters PicoTag system at 108°C for 20 h. After drying *in vacuo*, hydrolysates were examined on a Waters HPLC system with ninhydrin detection.

Differential scanning calorimetry. Collagen melting temperatures (T_m) were determined by DSC using a Mettler Toledo DSC 3000 instrument (Mettler, Schwerzenbach, Switzerland). Collagen samples were prepared at about 5 mg/ml, and equilibrated in 50 mM acetic acid, pH 2.8, by dialysis. Samples, about 100 mg of solution, were examined at a temperature increase of 1°C/min. Collagen T_m values are given as the temperature at the mid-point of the thermal transition.

Scanning electron microscopy. Collagen samples as provided, or after dilution to equal concentrations of 3 mg/ml with milliQ water, were coated on clean glass coverslips and air dried at room temperature in the clean air flow of a laminar flow hood. Dried samples were then gold coated and examined in a Philips XL-30 microscope.

RESULTS AND DISCUSSION

GENERAL SOLUTION PROPERTIES

There are a wide variety of solution measurements that can be readily made on collagen preparations (Table I). These include analysis for toxic metal components such as arsenic and lead, whose presence would make a preparation unsuitable for cosmetic application.

Table I
Analysis Results on Three Samples of Collagen Suitable for Cosmetic Applications

Test	AteloHelogen®	CLR Collagen®	Collasol®
Protein content (%w/v)	1.05%	0.28%	4.00%
pH	4.7	3.8	4.2
pI	>8.9	>8.9	<8.9
Arsenic	<1 ppm	<1 ppm	<1 ppm
Heavy metals	<5 ppm	<5 ppm	<5 ppm
Dry weight	1.15%	5.07%	5.25%
Conductivity	0.45 ms	19.0 ms	42.0 ms
Ash content	<0.1%	1.2%	1.1%
Hydration regain	21%	3%	7%

In the present study, the levels of these components were below the detection limit for all three samples.

An important feature of a collagen product is its collagen protein concentration. For fully soluble and essentially pure collagen preparations with concentrations above about 2–3 mg/ml (or solutions after clarification by centrifugation), a Biuret assay (16) against appropriate collagen standards is simple and rapid. However, non-collagenous impurities could interfere with this determination, leading to higher values. Total nitrogen content can also be readily determined by Kjeldahl analysis. This value allows the collagen concentration of the sample to be estimated, using a conversion factor based on the known structure of collagens (17). This factor is dependent on the collagen type and the species of origin. Again, this method can give erroneous values for collagen content if any non-collagenous proteins are present. Hydroxyproline is found as an amino acid that is characteristic of collagenous proteins, and therefore provides a specific method for determination of collagen content, separate from any other proteins that may be present. Hydroxyproline content and the total amino acid composition can be determined by amino acid analysis (see below). Hydroxyproline content can also be determined by a specific colorimetric assay (18).

The use of dry weight is not suitable to determine collagen content, as some preparations contain a significant salt content, as can be seen from the ash content of samples (Table I). The conductivity of a sample can confirm the presence of salts (Table I). For example, AteloHelogen® shows a negligible ash content, and has a very low conductivity, whereas both CLR Collagen® and Collasol® both have a significant ash content and significant conductivities, although these measures are not necessarily linked, as they depend on the nature of the salts present (Table I).

The pH values for the preparations examined in this study varied (Table I). These values may be important in developing formulations. The presence of salts may affect the pH, as they may be buffering the collagen solution (Table I). Samples of collagen that contain oligomers are more soluble under low pH conditions—at higher pH the collagen oligomers become less soluble and may precipitate (10). On the other hand, at a higher pH, such as neutral pH, a minimum salt content of around 0.15 M NaCl is needed to ensure collagen solubility (10). Thus, the higher pH of the AteloHelogen® collagen with a very low salt content (Table I) indicates a highly monomeric collagen preparation.

WATER REGAIN

The intrinsic water-binding property of collagen is critical to its excellent performance

as a humectant in cosmetic preparations. An estimate of this water-binding capacity may be obtained from the water regain by dry samples held under constant humidity (32%) (Table I). It can be seen that all collagen samples in the present study showed water regain after drying, but the extent or rate varied between the samples for the present method at 32% relative humidity. When samples were held at 87% relative humidity (saturated Na_2CO_3), all absorbed sufficient water to form solutions or wet slurries of collagen (data not shown). The variation between samples could reflect the presence of salts. The conductivity and ash content data (Table I) show that AteloHelogen® has a very low salt content, whereas the other two samples both contain salts. The nature of the salts (which was not determined) could appear to increase the collagen water regain if they are particularly hygroscopic, but this is not apparent in the present study. Alternatively, they could reduce the water regain, by slowing down the rate at which equilibrium is attained, for example. An alternative approach to compare samples would be to examine them after extensive dialysis to remove any salts present.

SPECTROSCOPY

Commercial collagen preparations are usually colorless solutions or they may be white if there is any insoluble material present. The principal use, therefore, of UV/visible spectroscopy is not to detect colored impurities, but rather to assess the level of turbidity that may be present in a soluble collagen preparation. For clear samples, it may also be used to detect and potentially quantitate any UV-absorbing preservatives that may be present. Material may be assessed as supplied or after dilution, for example, either in water so as to minimize changes in pH or in dilute acetic acid so as to ensure the solubility of non-cross-linked components, as in certain preparations it is possible that soluble collagen has formed into fibrils at neutral pH.

As an illustration, comparison of the three test collagens shows that AteloHelogen® was particularly transparent as supplied, whereas both CLR Collagen® and Collasol® were turbid (Figure 1A). After dilution in water to equivalent concentrations of 1 mg/ml, the clarity of Collasol® was still poor, while that for CLR Collagen® was significantly improved, although still much less than AteloHelogen® (Figure 1B). The solubility of Collasol® was improved in acetic acid (data not shown).

IR spectroscopy (Figure 2) provides a method for showing protein identity in the sample. It may also show the presence of organic buffers or preservatives. IR spectroscopy also has potential for assessing the extent of collagen denaturation in a collagen preparation, as any gelatine present changes the relative intensity of the bands at 1660 cm^{-1} and 1633 cm^{-1} (19). However, this requires high resolution. In the present study, the resolution of the IR spectra was not sufficient, and also seemed to vary between preparations (Figure 2). Thus IR spectroscopy may not be a convenient method for estimating the content of denatured collagen (gelatine) present in a sample. The collagen/gelatine content of fully clear solutions may otherwise be examined by ORD or CD spectroscopy, where standard values have been reported (20,21), but this requires access to specialized equipment. Sample clarity is an issue: insoluble collagen interferes and can be removed by centrifugation, but the resulting analysis is not representative of the total sample. Loss of gelatine components after brief proteolysis, where collagen is stable, followed by collagen precipitation, also provides a convenient method for estimating gelatine content.

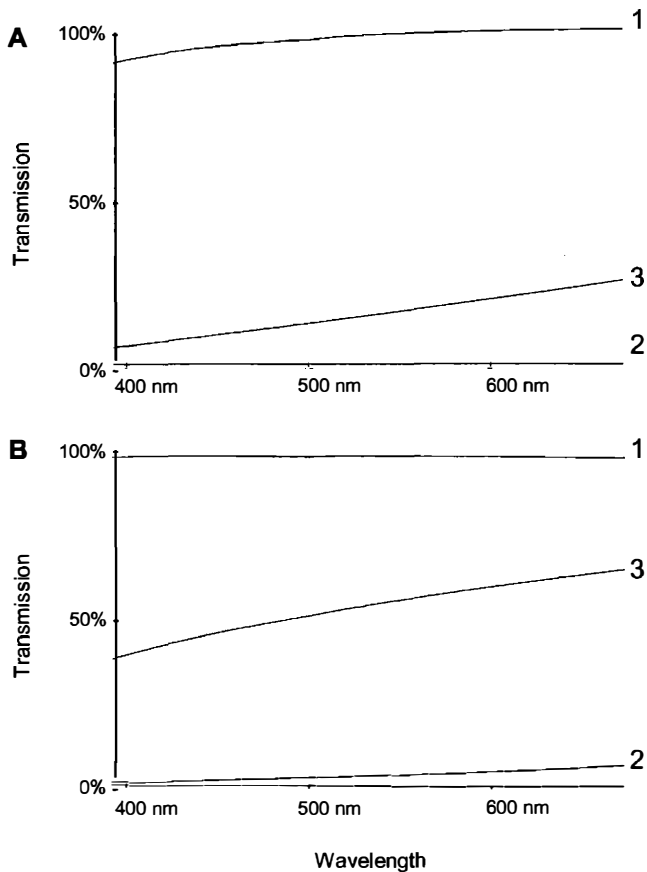


Figure 1. UV/visible spectra for three collagens for cosmetic use: (1) AteloHelogen[®], (2) Collasol[®], and (3) CLR Collagen[®] at the concentration supplied (A) and after dilution to equal concentrations of 3 mg/ml with water (B).

ELECTROPHORESIS

Various electrophoretic techniques are suitable for collagen analyses. These techniques are cheap and readily performed, particularly if commercially available pre-cast gels are used. In the examples, (Figures 3–5), the three different collagens for cosmetic use have been compared. Since the stain that is used to detect the collagenous components in each of the electrophoretic methods is not collagen-specific but stains all proteins, these electrophoretic methods also allow any protein contaminants (serum albumin, for example) to be detected in collagen samples.

In SDS-PAGE (Figure 3), the molecular weight distribution of the individual chain components after denaturation is shown. The single-chain components, the α -chains, are the fastest moving components from intact collagens. These chains show that AteloHelogen[®] contains type III collagen, while this is absent from CLR Collagen[®]. Collasol[®] shows an atypical chain pattern, which also prevents assessment of any type III collagen present. None of the collagens show any significant bands moving faster than the α -chains; if present, these bands could indicate degradation products, although they

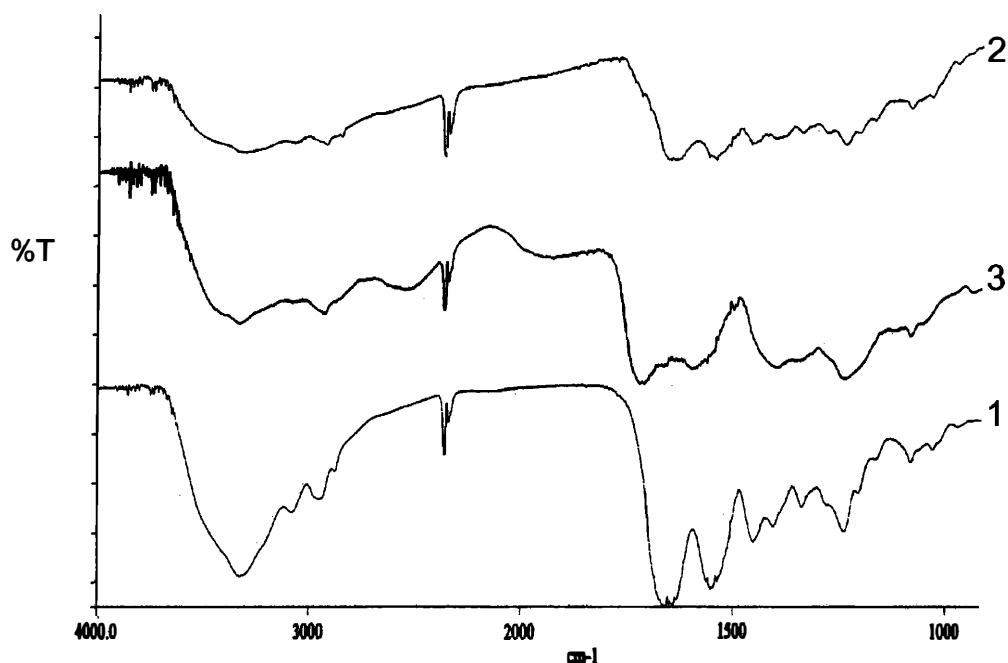


Figure 2. Infra-red spectra for three collagens for cosmetic use: (1) AteloHelogen®, (2) Collasol®, and (3) CLR Collagen®.

may also occur if the sample is heated in sample buffer for >2 min prior to electrophoresis. Crosslinked chain components, the dimer β - and trimer γ -chains, for example, move more slowly on the gel. These indicate (Figure 3) that AteloHelogen® has fewer crosslinked components than the other collagens. The bands that are present are those normally shown by purified type I and type III collagens (22), and no bands due to other collagen types are evident. Again, the pattern shown by the Collasol® is complex and atypical, with a high proportion of crosslinked material, much of which is too large to enter the gel.

Electrophoresis can also be performed under non-denaturing conditions, and uses highly porous gels to allow the larger collagen molecules to migrate (14). This system (Figure 4) shows the soluble collagen that is present in a preparation. Collagen dimers also enter the gel, but insoluble or highly polymeric material does not enter. In the example (Figure 4), it can be seen that all three samples contain soluble monomeric collagen of native size. AteloHelogen® shows fewer crosslinked dimer components than CLR Collagen®. Collasol® shows a poor, streaky pattern, with less collagen behaving as a soluble component. This suggests that much of this collagen may be crosslinked or have been modified during preparation. If the isoelectric point is low (see below), then material may not enter the gel as readily at pH 3.1.

Gel permeation chromatography can also be used to examine the molecular weight distribution of soluble material in a collagen preparation (23). However, this requires specialized equipment and columns. Also, as all insoluble material must be removed prior to analysis so that it does not block the flow in the column, the data are not representative of the complete sample. Electrophoresis provides a simpler, better method.

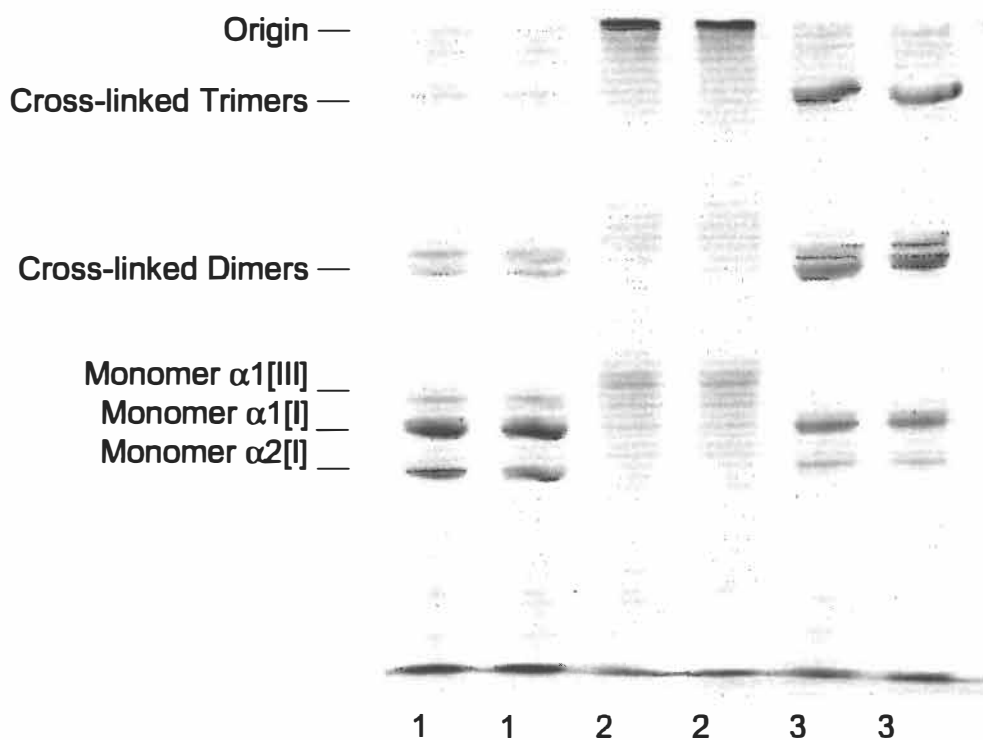


Figure 3. SDS-polyacrylamide gel electrophoresis of three collagens for cosmetic use: (1) AteloHelogen[®], (2) Collasol[®], and (3) CLR Collagen[®].

Electrophoresis can also provide a simple method to estimate the isoelectric point of the collagen preparation. Native collagens have a high pI, typically above pH 9 (24), consistent with the determined amino acid sequence data (17), whereas collagen that has been prepared from alkali-treated material (as a by-product of liming during leather making, for example) will have been deamidated and will have a low pI value (24).

The migration of native collagen, towards anode or cathode, is an indication of its net charge at a given pH. For example (Figure 5), collagens that migrate with a net positive charge at pH 9.0 have high pI values typical of native collagens. Thus, the present data indicate that both AteloHelogen[®] and CLR Collagen[®] have the high pI values found for native, unmodified collagen, whereas Collasol[®] shows a low pI, such as may be found from lime-treated hide collagen.

AMINO ACID ANALYSIS

Amino acid analysis data are presented for the three different collagen samples (Table II). This technique is readily available through analytical service providers if it is not available in-house. These data show that all samples have the high Gly content, around one third of all amino acids, that is a characteristic of collagens. If a large amount of protein impurity is present, this value may become lower, but for most samples this type of analysis will not allow accurate determination of small quantities of impurity. These

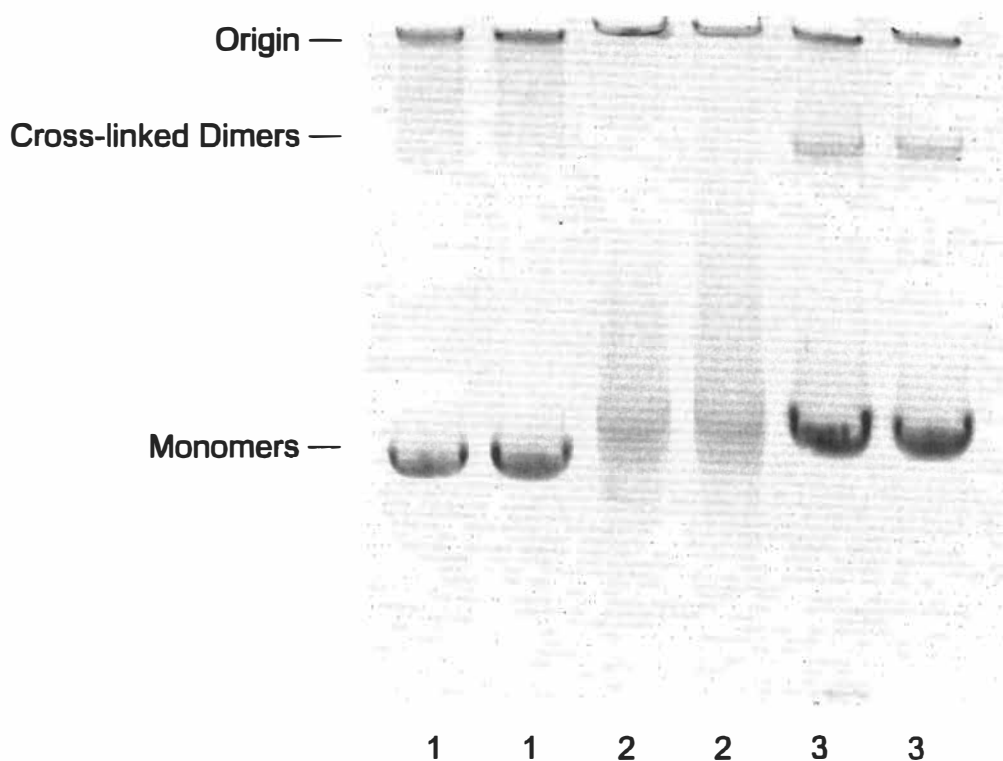


Figure 4. Native (lactic acid pH 3.1) polyacrylamide gel electrophoresis of three collagens for cosmetic use: (1) AteloHelogen®, (2) Collasol®, and (3) CLR Collagen®.

data also show the high content in Pro and Hyp, around one fifth of all amino acids, that are essential elements of the collagen structure. The avian collagen, AteloHelogen®, which is more thermally stable (see below), shows an increased Pro and Hyp content. The amount of Tyr present shows the extent to which the telopeptides have been removed, as this amino acid is generally only found in these regions in type I collagen. However, type III collagen does contain a low level of Tyr within the helical domain (17); if analysis shows that this collagen type is present, this must be taken into account (see below). Of the three collagens examined, AteloHelogen®, which is described as monomeric, shows a very low Tyr content, despite the type III collagen shown by electrophoresis (Figure 3).

Amino acid analysis also provides an approach to quantitation of the collagen preparations. These data may be obtained by using the Hyp content, and relating this value to the known amount of this amino acid in the species of the collagen (17). Alternatively, the yields of all amino acids can be determined and summed. A key issue in determining collagen content is the water content that is present. Certain water molecules form an integral part of the structure and cannot be readily removed from native collagens by drying. Other water present is more loosely bound; the amount of this water is dependent on the relative humidity of the environment and so presents significant difficulties in collagen quantitation if weighing of dry samples is needed. The use of amino acid analysis allows a direct measure of the collagen content of a collagen solution.

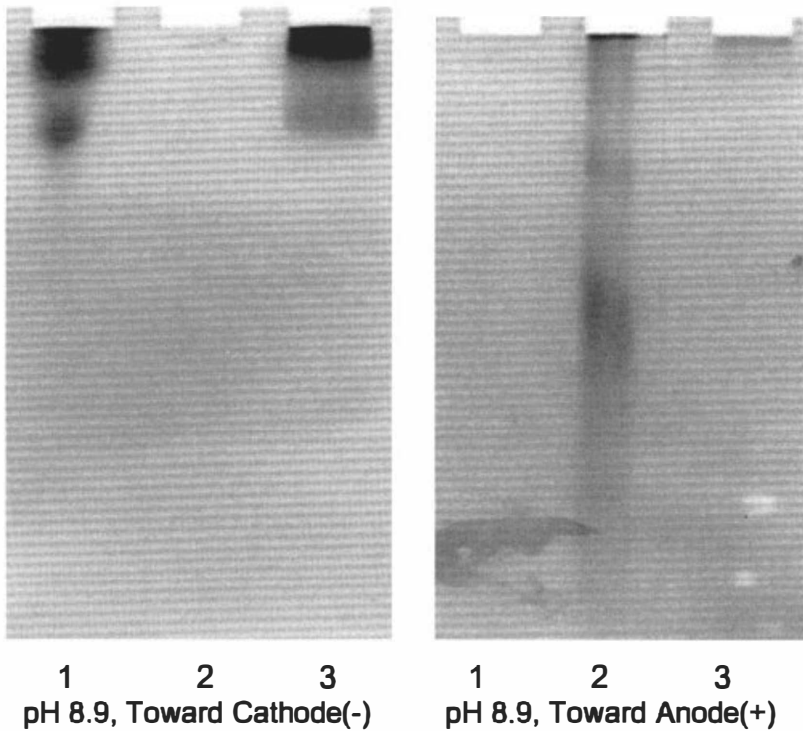


Figure 5. Estimation of pI for three collagens for cosmetic use from electrophoretic mobility at pH 8.9: (1) AteloHelogen®, (2) Collasol®, and (3) CLR Collagen®.

DIFFERENTIAL SCANNING CALORIMETRY

Various methods have been described for determining the thermal stability of collagens, where the change in a parameter is followed with increasing temperature. These include spectral methods, such as UV spectroscopy (25), ORD (20), and CD (21) enzyme digestion susceptibility (26), and calorimetric methods, such as differential scanning calorimetry (DSC) (27,28).

The results obtained can depend on the method used. For example, even for any single method, different results may be obtained by changing the rate of heating. Also, the method used for calculating and reporting thermal stability is important. For example, most correctly report thermal stability as the midpoint of the thermal transition, as in the present report. However, others, typically measuring preparations with lower stability, may report the higher temperature at the end of the transition. Thus, thermal stability must be compared using defined standard conditions.

In the present study we have examined the thermal stability of a range of collagens, including those available for cosmetic formulation, by a single method, DSC, using acetic acid as the solvent to ensure sample solubility. This method was chosen because it is generally readily available and has proven reproducibility. Data are reported using the generally accepted method of the midpoint of the thermal transition.

All collagens gave distinct single endothermic transitions by DSC (Figure 6), with similar transition energies for comparable quantities of collagens. The thermal transi-

Table II
Amino Acid Analysis Results for Three Different Collagen Preparations

Amino acid	AteloHelogen®	CLR Collagen®	Collasol®
HOPro	98	73	83
Asp	42	48	44
Thr	17	19	17
Ser	22	31	28
Glu	73	80	80
Pro	125	121	123
Gly	347	325	342
Ala	114	112	113
Cys	n.d.	n.d.	n.d.
Val	19	25	26
Met	n.d.	n.d.	n.d.
Ile	11	14	13
Leu	24	31	29
Tyr	1	7	3
Phe	13	16	14
His	4	6	4
HOLys	n.d.	n.d.	n.d.
Lys	28	31	26
Arg	54	55	50
Trp	n.d.	n.d.	n.d.

Data are expressed as residues per 1000. HOPro: 4-hydroxyproline. HOLys: hydroxylysine. Amino acids sensitive to oxidation (Met and Cys) were not determined. n.d.: not determined.

tions allowed the determination of T_m values (Table III). Replicate determinations were reproducible to 0.3°C or better.

Significant variation in melting temperature was evident among the collagens from different species (Table III). Comparison of type I collagen T_m values shows that chicken collagen was the most stable at 44.7°C, about 2.5°C more stable than the mammalian samples, which had T_m values in a range between 42.0°C and 42.2°C. The fish collagens were considerably less stable (Table III), consistent with the previous observation that collagen stability approximates the upper environmental temperature experienced by an organism (8). Thus *Macruronus* lives in cold waters (29), giving it a lower environmental temperature than *Tilapia*, which lives in tropical regions where farmed fish may have environmental temperatures around 30°C (30). The *Tilapia* collagen, which has been suggested as suitable for cosmetic applications, gave a T_m of 35.8°C (Table III). There is some variation of T_m with the pH of the samples, and so the pH of cosmetic collagen samples needs to be considered if the solutions are not made up in a standard solvent.

The melting data show that for a given species, the type III collagen had a slightly greater T_m value, about 1.2°C higher for mammals and 0.4°C higher for chickens, consistent with the stabilizing effects of a higher hydroxyproline content and the C-terminal disulfide bonds that are present in type III collagen (17,31). Although purified chicken type I and type III collagens show a small difference in T_m (Table III), AteloHelogen®, which contains a low level, about 7–10% of type III collagen (Figure 3, personal communication, Meddicoll), gave a single transition comparable to the purified chicken type I collagen, and a higher transition due to the type III component was not observed (Figure 6).

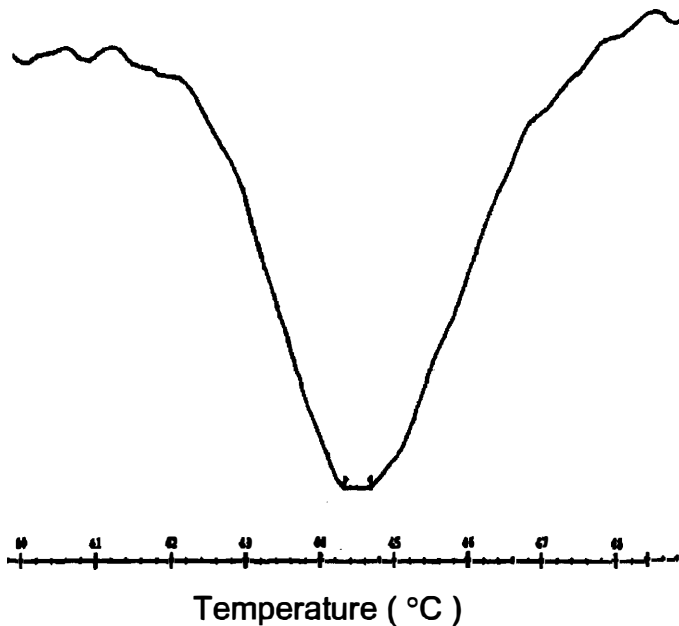


Figure 6. The DSC melting curve for AteloHelogen[®], showing a melting point determined from the midpoint of the transition at 44.6°C.

Table III
Melting Temperature Values Determined by DSC for Various Collagens

Collagen type	T _m pH 2.8 (°C)
Bovine I	42.2
Bovine III	43.5
Pig I	42.0
Pig III	43.2
Chicken I	44.7
Chicken III	45.1
Nile perch (<i>Tilapia</i> sp.)	35.8
Blue Grenadier (<i>Macruronus</i> sp.)	23.0
AteloHelogen [®]	44.6

Thus, for monomeric cosmetic collagens, the avian collagen, AteloHelogen[®], would have a greater stability than the bovine collagens, which may provide significant benefits for formulation and shelf-life stability. *Tilapia* collagen has been suggested for cosmetic applications, but it has a lower stability than the bovine and particularly the avian collagens.

SCANNING ELECTRON MICROSCOPY

SEM provides an approach to evaluating any fibrous or particulate material that may be present in a collagen preparation. The samples were dried down onto a clean flat surface for evaluation. In the examples (Figure 7), the AteloHelogen[®] (panel 1), which presented

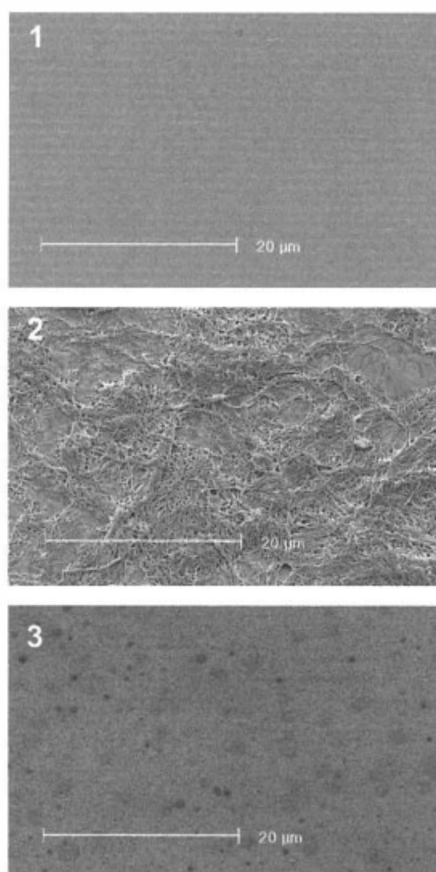


Figure 7. SEM images of thin films of three collagens for cosmetic use: (1) ArelolHelogen®, (2) Collasol®, and (3) CLR Collagen®. Scale bars are shown.

as a clear solution, showed an essentially featureless film, confirming the lack of particulate or fibrous components. On the other hand, the Collasol®, which was a white turbid sample, showed extensive fibrous structures (Figure 7, panel 2). Of interest was the CLR Collagen® sample, which, although presenting as a fairly clear solution, showed a range of black or grey zones under SEM (Figure 7, panel 3). The nature of these spots is not clear.

OTHER TECHNIQUES

A range of other techniques may also be valuable in assessing collagen for certain applications. These include determination of viscosity and solubility, over a range of pH values to match formulation requirements; density; refractive index; and lipid content. Microbiological content and immunological response may also be issues of concern.

CONCLUSIONS

The collagen samples examined in the present study show a wide range in various

properties. This range suggests that one of these collagens could probably be selected to meet specific manufacturing needs. The tests that have been described allow the potential collagens to be evaluated, and different batches to be compared for quality assurance. The tests that should be applied to samples will depend on the needs of the particular formulation being developed. A key property of collagens, their water-binding capacity, may also be measured, but this data needs to be treated with care, as the salt present in certain preparations can lead to false interpretations. Of the collagens examined, Atelo-Helogen[®] was monomeric and particularly pure, both biochemically and in the absence of salts.

The country of origin may be a consideration at present due to risk of any viral or prion-based contamination. Thus, non-bovine collagens may also be preferred as there may be less risk of disease transmission. In this respect, the chicken collagen, Atelo-Helogen[®], is intrinsically more stable than marine collagens. Consumer sensitivity to avian collagens is potentially low, and comparable to mammalian collagens despite the structural differences (data not shown). It has also been suggested that collagen could permeate intact skin and augment the collagenous tissue. This seems implausible, however, as collagen molecules would be too large and too well bound to penetrate the stratum corneum (32). Should collagen or fragments pass into the underlying tissue, they would be unable to participate in the complex biosynthetic pathway that characterizes collagen deposition in tissue (7). Evidence has been presented that confirms that this augmentation does not occur in skin with intact stratum corneum (33). Indeed, the lack of skin penetration should minimize sensitivity issues.

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