

Composition–viscosity relationship of silk fibroin / poly (vinyl alcohol) spinning solutions: a multiplicative model validated by replicate-based statistics

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Abstract

Dynamic viscosity is the single solution property that most strongly determines whether an electrospinning process is stable, yet composition–viscosity relationships for blended biopolymer systems are almost always described by additive polynomial models whose local accuracy is rarely checked. This work measures the dynamic viscosity of nine silk fibroin / poly(vinyl alcohol) composite spinning solutions on a rotational viscometer with ten replicates per sample, and uses the replicate structure to validate the resulting models rather than relying on the coefficient of determination alone. A conventional second-order polynomial fitted to a full 3^2 factorial design gives $R^2 = 0.977$ but proves locally inaccurate, with a mean relative approximation error of 18.6% and a maximum of 41.2%. Because the viscosity of polymer solutions above the entanglement threshold depends exponentially rather than linearly on concentration, a multiplicative model was constructed by logarithmic transformation of the response: $\ln v = 5.8390 + 0.1939X_1 + 0.9567X_2 + 0.0827X_1X_2 - 0.2472X_1^2 + 0.3270X_2^2$. This model gives $R^2 = 0.9977$ and a mean relative error of 3.2%, below the root-mean-square relative scatter of the experiment (6.2%); all six coefficients are significant by Student's test. Cochran's test rejects variance homogeneity ($G = 0.450 > 0.331$), the coefficient of variation rising from 0.28% in the low-viscosity samples to 12.55% in the high-viscosity ones, which is attributed to structuring of concentrated poly(vinyl alcohol) solutions during storage. The final composite formulation has a viscosity of 2139.6 ± 140.8 mPa·s against 3992.5 ± 20.8 mPa·s for the reference poly(vinyl alcohol) solution, and a minimum of 2000 mPa·s is established as the threshold for stable jet formation in this system.

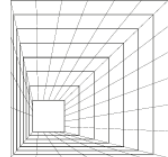
Keywords: dynamic viscosity; rotational viscometry; design of experiments; multiplicative model; Cochran criterion; heteroscedasticity; silk fibroin; poly(vinyl alcohol); electrospinning

1. Introduction

The stability of the electrospinning process is governed largely by the properties of the spinning solution, of which dynamic viscosity is the most important single indicator. An increase in viscosity corresponds to a higher polymer concentration and therefore to a higher mass yield per unit time; it damps the capillary waves that disrupt the liquid jet and increases its stability; and, through the molecular weight and structure of the polymer, it is linked to the rheological and strength characteristics of the solution and to its resistance to deformation loading and cavitation [1, 2].

Under ordinary conditions the dynamic viscosity of solutions intended for electrospinning is considered to lie between 60 and 7000 mPa·s, and most often between 100 and 3000 mPa·s [2]. This interval, however, is very wide, and for each specific polymer system the stability threshold must be established experimentally.

In the rheology of polymer solutions the dependence of viscosity on concentration is not linear: above the entanglement concentration it approaches an exponential law and is expressed as $\eta \approx \eta_0 \cdot \exp(k \cdot c)$ [3, 4]. This indicates that multiplicative (logarithmic) models are more appropriate than simple polynomial approximations when composition–viscosity relationships are to be modelled over a wide



range; nevertheless, in the electrospinning literature the additive polynomial approach predominates, and model adequacy is usually judged by the coefficient of determination alone.

The coefficient of determination is, however, a poor guide to local accuracy when the response spans an order of magnitude, because it is dominated by the ability of the model to reproduce the overall trend. A model may account for 98% of the variance and still misrepresent individual design points by tens of per cent — a discrepancy that matters directly when the model is used to select a formulation close to a stability threshold.

The novelty of this work lies in constructing a multiplicative composition–viscosity model for a silk fibroin / poly(vinyl alcohol) system and in demonstrating its superiority over the conventional polynomial model by statistical criteria based on replicate measurements rather than on the coefficient of determination.

The aim of the work is to determine experimentally the dynamic viscosity of composite spinning solutions, to construct a statistically validated composition–viscosity model, and to establish the viscosity threshold required for stable electrospinning of this system.

2. Materials and methods

2.1. Solutions

Nine composite spinning-solution samples were prepared from a silk fibroin solution (in a solvent based on calcium chloride, distilled water and ethanol) and a 16% aqueous solution of poly(vinyl alcohol), Arkofil PPL grade. The samples were prepared according to a full 3^2 factorial design. The factors were the fibroin mass per 5.5 g of solvent, X_1 (0.10; 0.15; 0.20 g), and the share of the 16% poly(vinyl alcohol) solution, X_2 (20; 30; 40%); the corresponding coded values were -1 , 0 and $+1$.

2.2. Viscometry

Dynamic (absolute) viscosity was measured with an RM 100 Plus rotational viscometer (Lamy Rheology Instruments, France). Its principal technical characteristics are given in Table 1 and its general view in Fig. 1.

Table 1. Principal technical characteristics of the RM 100 Plus rotational viscometer.

Characteristic	Value
Instrument type	rotational viscometer
Rotor speed	0.3–1500 min ⁻¹
Temperature measurement	built-in PT100 sensor, -50 to $+300$ °C
Torque	0.05–30 mN·m
Measurement error	$\pm 1\%$ of full scale
Repeatability	$\pm 0.2\%$
Viscosity range	1–540 000 000 mPa·s

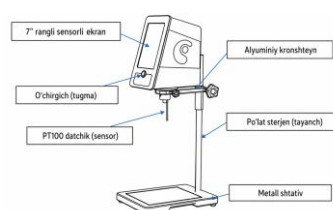
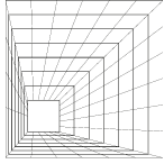


Fig. 1. General view of the RM 100 Plus rotational viscometer.

The operating principle is based on the dependence of the torque applied to a rotor immersed in the medium on the viscosity of that medium. The torque acting on the rotor is



$$Mk = F \cdot r, \quad (1)$$

where F is the force applied to the rotor (N) and r the rotor radius (m). In accordance with Newton's law,

$$F = \eta \cdot \omega \cdot S / l, \quad (2)$$

where ω is the angular velocity of the rotor (s^{-1}), S the working area of the rotor (m^2), l the gap between cup and rotor (m) and η the dynamic viscosity ($Pa \cdot s$). Substituting Eq. (2) into Eq. (1) gives

$$Mk = \eta \cdot \omega \cdot S \cdot r / l, \quad (3)$$

so that for a fixed rotor geometry and angular velocity the torque is directly proportional to the viscosity.

2.3. Statistical treatment

Each sample was measured $n = 10$ times. The statistical treatment followed the sequence: (a) the mean, sample standard deviation, coefficient of variation and 95% confidence interval of the mean were computed for each design point; (b) homogeneity of variances was tested by Cochran's criterion; (c) the regression coefficients were estimated by least squares and their significance assessed by Student's test; and (d) the significance and adequacy of the model were assessed by Fisher's criterion and by the mean relative approximation error. All computations were carried out at a confidence level of 0.95.

Cochran's criterion was computed as

$$G = S^2_{max} / \Sigma S^2_i, \quad (4)$$

where S^2_{max} is the largest sample variance and ΣS^2_i the sum of the variances at all design points. The reproducibility variance S^2_r was taken as the mean of the variances at the design points, with $f = 9 \times (10 - 1) = 81$ degrees of freedom.

Because the response spans an order of magnitude, a multiplicative model was also fitted by applying least squares to the logarithm of the response, and the two models were compared on the original scale through the mean and maximum relative approximation errors.

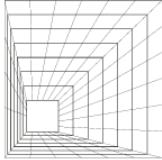
3. Results

3.1. Measured viscosities and their scatter

Table 3 gives the statistical indicators obtained from ten replicates at each of the nine design points.

Table 2. Individual viscosity measurements for the nine spinning solutions, mPa·s.

Replicate	1.1	2.1	3.1	1.2	2.2	3.2	1.3	2.3	3.3
1	126	182	164	224	413	285	619	1211	1375
2	126	182	163	224	426	286	665	1204	1356
3	125	181	163	223	404	302	764	1111	1321
4	126	182	164	223	326	312	673	1168	1332
5	124	181	163	223	307	311	771	1144	1318
6	126	182	164	224	313	376	710	1273	1329
7	126	182	163	223	400	275	810	1120	1341
8	126	181	163	221	324	285	773	1204	1336
9	127	182	164	223	345	309	788	1182	1323
10	126	181	164	224	384	298	812	1161	1330
Mean	125.8	181.6	163.5	223.2	364.2	303.9	738.5	1177.8	1336.1



The individual measurements are reported in full in Table 2 so that the variance structure used in the statistical treatment can be verified independently. Table 3 summarises them.

Table 3. Statistical indicators of the dynamic viscosity of the spinning solutions ($n = 10$).

Sample	X_1 , g	X_2 , %	Mean v , mPa·s	S , mPa·s	V , %	95% CI, $\pm$
1.1	0.10	20	125.8	0.79	0.63	0.56
2.1	0.15	20	181.6	0.52	0.28	0.37
3.1	0.20	20	163.5	0.53	0.32	0.38
1.2	0.10	30	223.2	0.92	0.41	0.66
2.2	0.15	30	364.2	45.70	12.55	32.69
3.2	0.20	30	303.9	28.38	9.34	20.30
1.3	0.10	40	738.5	67.20	9.10	48.07
2.3	0.15	40	1177.8	48.17	4.09	34.46
3.3	0.20	40	1336.1	17.53	1.31	12.54

The computed value of Cochran's criterion is $G = 0.450$, which exceeds the critical value at the 0.95 confidence level ($G_{cr} = 0.331$ for $k = 9$ and $f = 9$). The hypothesis of variance homogeneity is therefore rejected: the scatter is very small in the low-viscosity samples ($V = 0.28$ – 0.63%) and considerably larger in the viscous ones ($V = 9$ – 12.55%). The reproducibility variance is $S^2_r = 1115.5$ (mPa·s) ^{2} and the root-mean-square relative scatter is 6.2%.

3.2. Polynomial model

At the first stage a conventional second-order polynomial was fitted:

$$v = 297.10 + 119.33 \cdot X_1 + 463.58 \cdot X_2 + 139.97 \cdot X_1 X_2 + 323.45 \cdot X_2^2. \quad (5)$$

Its coefficient of determination is $R^2 = 0.977$; the regression significance criterion is $F = 42.05 > F_{cr}(0.05; 4; 4) = 6.39$, and all coefficients are significant by Student's test ($t = 26.5$ – $107.5 > t_{cr} = 1.99$). Nevertheless the local accuracy of the model proved low: the mean relative approximation error is 18.6% and the largest individual error 41.2% (Table 4).

3.3. Multiplicative model

The cause of this shortcoming is a mismatch between the form of the model and the physics of the system. Because the viscosity of polymer solutions depends exponentially on concentration [3, 4], an additive polynomial cannot give satisfactory results over the range covered here (125–1336 mPa·s, a factor of 10.6). A multiplicative model was therefore constructed by logarithmic transformation of the response:

$$\ln v = 5.8390 + 0.1939 \cdot X_1 + 0.9567 \cdot X_2 + 0.0827 \cdot X_1 X_2 - 0.2472 \cdot X_1^2 + 0.3270 \cdot X_2^2, \quad (6)$$

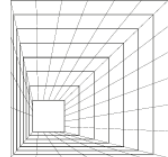
or, in explicit multiplicative form,

$$v = 343.9 \cdot \exp(0.1939 \cdot X_1 + 0.9567 \cdot X_2 + 0.0827 \cdot X_1 X_2 - 0.2472 \cdot X_1^2 + 0.3270 \cdot X_2^2). \quad (7)$$

The coefficient of determination of Eq. (6) is $R^2 = 0.9977$ and all its coefficients are significant ($t = 8.4$ – $399.1 > t_{cr} = 1.99$). Table 3 compares the agreement of the two models with the experimental data.

Table 4. Design matrix and comparative accuracy of the two models.

Sample	X_1	X_2	Experiment v , mPa·s	Eq. (5)	Error, %	Eq. (6)	Error, %
1.1	–1	–1	125.8	177.6	–41.2	127.8	–1.6
2.1	0	–1	181.6	157.0	13.6	183.0	–0.7



Sample	X ₁	X ₂	Experiment ν , mPa·s	Eq. (5)	Error, %	Eq. (6)	Error, %
3.1	+1	-1	163.5	136.3	16.6	159.7	2.3
1.2	-1	0	223.2	177.8	20.4	220.9	1.0
2.2	0	0	364.2	297.1	18.4	343.4	5.7
3.2	+1	0	303.9	416.4	-37.0	325.6	-7.1
1.3	-1	+1	738.5	824.8	-11.7	734.2	0.6
2.3	0	+1	1177.8	1084.1	8.0	1239.8	-5.3
3.3	+1	+1	1336.1	1343.4	-0.5	1276.7	4.4
Mean absolute relative error, %	—	—	—	—	18.6	—	3.2

The mean relative error of the multiplicative model (3.2%) is below the root-mean-square relative scatter of the experiment (6.2%). Eq. (6) therefore reproduces the experimental data within the limits of measurement uncertainty. The graphical interpretation of the relationship obtained is shown in Fig. 2.

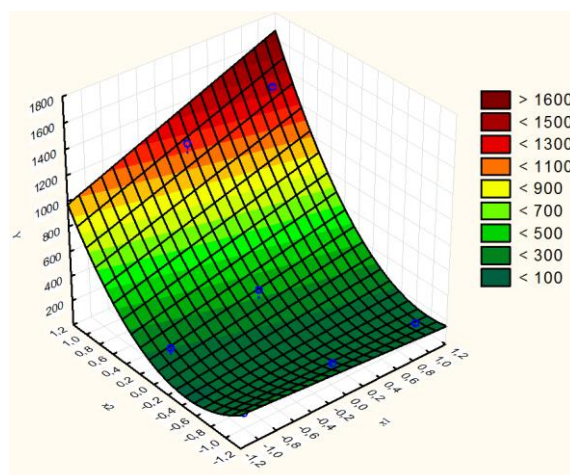


Fig. 2. Dependence of the dynamic viscosity of the solutions on their composition.

3.4. Final formulation and reference solution

Preliminary electrospinning trials were carried out with samples 1.3, 2.3 and 3.3, which had the highest viscosities. The process was nevertheless insufficiently stable, so the poly(vinyl alcohol) share was raised to 60% and a final composite formulation was prepared consisting of 40% silk fibroin solution (0.2 g of fibroin per 5.5 g of solvent) and 60% of the 16% poly(vinyl alcohol) solution. Table 5 gives the viscosity measurements for this formulation and for the pure 16% poly(vinyl alcohol) solution taken as a reference.

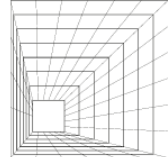


Table 5. Dynamic viscosity of the final composite formulation and of the 16% poly(vinyl alcohol) solution (n = 10).

Solution	Mean η , mPa·s	S, mPa·s	V, %	95% CI, $\pm$
Final composite formulation	2139.6	196.8	9.20	140.8
16% poly(vinyl alcohol) solution	3992.5	29.1	0.73	20.8

Comparison of the two variances by Fisher's criterion gives $S^2_{\text{comp}} / S^2_{\text{PVA}} = 45.6 > F_{\text{cr}}(0.025; 9; 9) = 4.03$, so that the reproducibility of the composite solution is significantly poorer than that of the reference. The difference between the means is highly significant by Welch's test ($t = -29.5$; $p < 0.001$).

4. Discussion

4.0. Concentration scaling and the entanglement regime

The multiplicative form of Eq. (6) invites a more fundamental test. If the solutions lie in the entangled semidilute regime, the zero-shear viscosity should follow a power law in the total polymer concentration, $\eta \propto c^n$, with n between about 3.75 and 4.7 depending on solvent quality [3, 19]. Expressing each of the nine formulations through its total polymer mass fraction — the sum of the poly(vinyl alcohol) and fibroin contributions, which ranges from 4.63 to 8.51 wt% — and regressing $\ln \eta$ on $\ln c$ gives

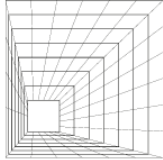
$$\eta \propto c^{(4.05 \pm 0.66)}, \quad r = 0.919, \quad p = 4.7 \times 10^{-4}. \quad (8)$$

The exponent lies squarely within the range predicted for entangled semidilute solutions and its confidence interval comfortably brackets both the good-solvent value of 3.75 and the theta-solvent value of $14/3 \approx 4.67$. This is an independent confirmation, obtained without any adjustable parameter, that the composite solutions are entangled rather than semidilute-unentangled, and it supplies the physical justification for preferring a multiplicative model: an exponential dependence on the coded factors is the natural coded-variable representation of a power law in concentration.

The same analysis quantifies the asymmetry between the two polymers. Regressing on the poly(vinyl alcohol) concentration alone gives an exponent of 2.68 with $r = 0.926$, that is a comparable fit on a single variable. Fibroin therefore contributes to the total concentration without contributing proportionately to the entanglement network, which is consistent with a molecular weight too low, or a chain architecture too compact, to entangle at these concentrations. The regression on total concentration thus slightly over-weights the fibroin contribution, and this shows in extrapolation: applying Eq. (8) to the final formulation at $c = 11.0$ wt% predicts 2972 mPa·s against the 2139.6 mPa·s measured, an over-prediction of 39%.

That discrepancy is itself informative rather than merely a failure of extrapolation. The final formulation differs from the fitted series not only in total concentration but in composition: its poly(vinyl alcohol) fraction is 9.6 wt% against 6.4 wt% at the highest point of the design, while its fibroin fraction falls to 1.40 wt%. Since only the poly(vinyl alcohol) participates effectively in entanglement, a single-concentration power law cannot capture a change in the ratio of the two components, and the direction of the error — over-prediction when the non-entangling component is diluted — is exactly what that interpretation requires.

The structure of Eq. (6) makes the mechanism by which composition governs viscosity explicit. The coefficient of X_2 (the poly(vinyl alcohol) share) is 0.9567, almost five times that of X_1 (the fibroin mass, 0.1939): viscosity is in practice determined by the poly(vinyl alcohol) concentration. The positive quadratic term in X_2 (0.3270) indicates that viscosity grows at an accelerating rate as the poly(vinyl alcohol) share increases, while the negative quadratic term in X_1 (−0.2472) indicates saturation of the fibroin effect. The interaction term (0.0827) reflects the dependence of the fibroin effect on the poly(vinyl alcohol) concentration.



This structure agrees fully with the concentration analysis of the same system. The fibroin fraction in the solutions is 1.07–2.81 wt%, which is below the entanglement concentration, so its contribution to viscosity is limited; poly(vinyl alcohol), at 3.2–6.4 wt%, forms the entanglement network and acts as the viscosity-determining component. Quantitatively: at a 20% poly(vinyl alcohol) share, raising the fibroin mass from 0.10 to 0.20 g changed the viscosity only from 125.8 to 163.5 mPa·s (a factor of 1.3), whereas at 40% the same change raised it from 738.5 to 1336.1 mPa·s (a factor of 1.8).

The rejection of Cochran's criterion is also informative rather than merely inconvenient. In the low-viscosity samples the measurement scatter is at the level of the instrument's own error ($V < 0.7\%$), whereas in the viscous samples it rises to 4–12.5%. This is attributable to the thixotropic behaviour of concentrated polymer solutions and to their structuring during storage: in aqueous poly(vinyl alcohol) solutions the number of hydrogen bonds between macromolecules increases with time and supramolecular structures develop [5, 6]. The practical consequence is that viscous spinning solutions should be used shortly after preparation and that the number of replicate measurements for them should be increased.

Heteroscedasticity also has a methodological consequence for model validation. The classical Fisher adequacy test requires the reproducibility variance to be the same at all design points; when that requirement fails, the test loses its formal basis. In this work adequacy was therefore assessed by comparing the mean relative approximation error with the experimental scatter: $3.2\% < 6.2\%$ for Eq. (6) against $18.6\% > 6.2\%$ for Eq. (5). The multiplicative model is thus not only more accurate but also methodologically more appropriate, and the comparison illustrates a more general point — that a coefficient of determination of 0.977 can coexist with local errors exceeding 40%.

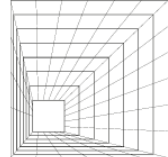
The experimental results showed that the viscosities of samples 1.3, 2.3 and 3.3 (738.5, 1177.8 and 1336.1 mPa·s respectively) were insufficient for stable electrospinning, whereas the final formulation at 2139.6 mPa·s gave a stable process. A minimum dynamic viscosity of 2000 mPa·s is therefore proposed as the threshold for stable jet formation in fibroin-containing solutions of this type. This value lies in the middle of the general interval quoted in the literature (60–7000 mPa·s) and is considerably above the values typical of the dilute polyester solutions used in most tissue-engineering studies, which explains why such solutions also require much lower field strengths.

The study has three principal limitations. First, the model is expressed in coded variables and is therefore applicable only within the factor ranges studied (fibroin 0.10–0.20 g, poly(vinyl alcohol) solution share 20–40%); extrapolation is particularly hazardous because of the exponential structure of Eq. (7). Second, temperature was not included as a factor, although the viscosity of polymer solutions follows an Arrhenius dependence on temperature. Third, the solutions may exhibit non-Newtonian behaviour, but since the measurements were made at a single rotor speed for each sample, the dependence on shear rate was not determined. All three define the directions of further work.

4.1. Viscosity is necessary but not sufficient for process stability

An apparent paradox in the present data deserves comment. The reference poly(vinyl alcohol) solution has a viscosity of 3992.5 mPa·s, nearly twice that of the final composite formulation, and both spin stably; yet samples 2.3 and 3.3, at 1177.8 and 1336.1 mPa·s, do not. Viscosity is therefore a necessary but not a sufficient condition, and the threshold identified here is specific to the fibroin-containing system rather than universal.

Two factors account for this. The first is the ionic strength contributed by the calcium chloride of the fibroin solvent, which raises the electrical conductivity of the composite solutions and alters the charge density carried by the jet; solutions of equal viscosity but different conductivity do not behave identically under the same field. The second is that viscosity in these composites is contributed almost entirely by the carrier polymer, whereas the fibroin fraction contributes solute that does not participate in the entanglement network. A composite at 1336 mPa·s therefore has a weaker entanglement network than a single-polymer solution at the same viscosity, which is consistent with the observation that the threshold has to be exceeded by a considerable margin before the process becomes stable.



The practical implication is that a viscosity threshold transferred from one polymer system to another should be treated as an order-of-magnitude guide only, and that conductivity should be measured and reported alongside viscosity whenever a salt-containing solvent is used.

4.2. Comparison with reported viscosities of silk fibroin systems

Reported viscosities for electrospinnable regenerated silk fibroin solutions vary widely because they depend on the solvent, the concentration and the degumming history of the fibroin, the last of which determines its molecular weight. Studies using formic acid or hexafluoroisopropanol typically operate at fibroin concentrations of 8–15 wt%, an order of magnitude above those attainable in the aqueous calcium chloride system used here, and correspondingly do not require a carrier polymer [9, 15].

The comparison identifies the real cost of choosing a non-toxic solvent. The calcium chloride system was selected because its residues are pharmacologically benign, but it limits the attainable fibroin concentration to 1.79–3.51 wt%, which in turn makes a carrier polymer unavoidable and dilutes the fibroin in the finished web. Raising the attainable fibroin concentration within a benign solvent system — for example by reducing the degumming severity so as to preserve molecular weight — would therefore address the dilution problem at its source and is the most valuable direction for subsequent work.

5. Conclusions

The dynamic viscosity of nine silk fibroin / poly(vinyl alcohol) composite spinning solutions was determined with ten replicates per sample; the values range from 125.8 to 1336.1 mPa·s and the coefficient of variation rises from 0.28% to 12.55% across the design.

Cochran's criterion rejects the homogeneity of variances ($G = 0.450 > G_{cr} = 0.331$); the increase in scatter is attributed to the structuring of concentrated solutions, which implies that viscous spinning solutions should be used shortly after preparation.

A conventional second-order polynomial model, despite a coefficient of determination of 0.977, was shown to have poor local accuracy, with a mean relative error of 18.6% and a maximum of 41.2%.

A multiplicative model based on the exponential nature of polymer-solution viscosity was proposed: $v = 343.9 \cdot \exp(0.1939X_1 + 0.9567X_2 + 0.0827X_1X_2 - 0.2472X_1^2 + 0.3270X_2^2)$. It gives $R^2 = 0.9977$ and a mean relative error of 3.2%, that is within the experimental scatter of 6.2%, and all its coefficients are significant.

The final composite formulation has a dynamic viscosity of 2139.6 ± 140.8 mPa·s against 3992.5 ± 20.8 mPa·s for the 16% poly(vinyl alcohol) reference, and a minimum of 2000 mPa·s is established as the threshold for stable electrospinning of this system.

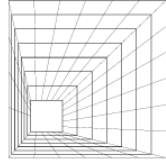
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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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