ARL-TR-10310 • MAR 2026

Quantifying Desiccation in Glycerol–Agarose Hydrogels

by Ella Willetts, W. David Hairston, and Christopher G. Sinks

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ARL-TR-10310 ● MAR 2026

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Ella Willetts
Energetics Technology Center

W. David Hairston and Christopher G. Sinks
DEVCOM Army Research Laboratory

REPORT DOCUMENTATION PAGE

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED	
		START DATE	END DATE
March 2026	Technical Report	December 2024	February 2025

4. TITLE AND SUBTITLE
Quantifying Desiccation in Glycerol–Agarose Hydrogels

5a. CONTRACT NUMBER	5b. GRANT NUMBER	5c. PROGRAM ELEMENT NUMBER
W911NF-23-3-0003		
5d. PROJECT NUMBER	**5e. TASK NUMBER**	**5f. WORK UNIT NUMBER**

6. AUTHOR(S)
Ella Willetts, W. David Hairston, and Christopher G. Sinks

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
DEVCOM Army Research Laboratory ATTN: FCDD-RLA-FC Aberdeen Proving Ground, MD 21005	ARL-TR-10310

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES
ORCIDs: Christopher G. Sinks, 0009-0003-5693-0274; W. David Hairston, 0000-0003-4432-8430

14. ABSTRACT
Electromagnetic (EM) radiation-emitting devices are becoming more common in the world and on the battlefield with the use of radar, high-power radios, and other nonionizing radiation-emitting devices. We lack an efficient way to test safety and biological effects of EM, so we need admittance-matching tissue simulants to test for detrimental effects and develop protective measures. Some of the best candidates for the simulants are hydrogels due to their high-water content and tissue-similar mechanics. However, these gels can desiccate over time, changing their properties over the course of experimentation. A common solution is to add a humectant, which helps keep water bound in the hydrogel. In this work, we quantify how much of a common humectant, glycerol, changes equilibrium water content across a series of agarose-based hydrogels kept under strict environmental conditions. From this data, we can estimate how much glycerol is required to stabilize a tissue-simulant model under a given set of environmental conditions, allowing for better testing of EM response of tissue over a longer period of time.

15. SUBJECT TERMS
Humans in Complex Systems, Hydrogels, Desiccation, Agarose, Tissue Simulant, Humectant

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
a. REPORT	b. ABSTRACT	C. THIS PAGE		
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UU	21

19a. NAME OF RESPONSIBLE PERSON	19b. PHONE NUMBER *(Include area code)*
Christopher G. Sinks	(520) 691-5358

STANDARD FORM 298 (REV. 5/2020)
Prescribed by ANSI Std. Z39.18

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Acknowledgments

This work was supported in part by the Energetics Technology Center under contract no. W911NF-23-3-0003.

1. Introduction

Electromagnetic (EM) radiation-emitting devices are becoming more common in the world and on the battlefield with the use of radar, radios, and other nonionizing radiation-emitting devices. We lack an efficient way to test safety and biological effects of EM, so we need to discover, invent, or research additional testbeds that are repeatable and reliable. Novel to routine—including medical imaging, communications, directed energy systems, and countless other technologies—these devices require testing that mimic the mechanical and dielectric properties of human skin, brain, bone, and other tissue. Cadavers offer one possible solution; however, their difficult acquisition, rapid biological deterioration, and their need for and change in properties due to preservation make them unreliable and impractical.[1] To relieve this issue, synthetic tissue phantoms emerge as a tool to safely and repeatably test and evaluate EM-emitting devices.[2]

Hydrogels are hydrophilic polymer networks that are characterized by their ability to swell and absorb large amounts of water. They can maintain their structural integrity while intaking large amounts of fluid, which makes them highly viable for drug delivery, soft robotics, tissue engineering, and other biomedical applications.[3] The water content in hydrogels directly impacts their mechanical and electrical properties, so naturally maintaining consistent content over time is critical. Desiccation is the process by which water gradually evaporates or leeches due to surrounding environmental factors like temperature and relative humidity (RH). These conditions can lead to changes in stiffness, shrinkage, or even water absorption and swelling under different environments. Desiccation of hydrogels in phantom head models can quickly alter the performance and repeatability, so if we can slow or prevent this process, we can develop robust surrogate models that can be used for repeated testing without losing fidelity to biological tissue properties. We can then use these types of models to better evaluate EM effects on tissue.

Hydrogels comprising agarose, glycerol, and water seem to match necessary characteristics, including Young's modulus, yield strength, permittivity, and impedance, that make them good candidates for human tissue models.[4] Hydrogels can be synthesized from a broad range of polymers[3]; for this study we use agarose, which is a polysaccharide extracted from seaweed commonly used for its biocompatibility and mechanical strength. Glycerol acts both as a humectant, a substance that increases water retention, and plasticizer, which increases flexibility, that can be added to agarose and other hydrogels. Both combined with water create a basis for tissue-mimicking materials. This study investigates how hydrogels made of agarose, glycerol, and water respond to desiccation under different concentrations of each material and environmental changes. Tracking water loss

and reabsorption across varying levels of controlled humidity and temperatures should allow us to further understand how to refine hydrogel composition for use in open-air applications for extended lengths of time. Our goal is to optimize a skin-like layer for use in a full-scale human head model to support safe and repeatable testing of emerging defense technologies.

2. Materials and Methods

Agarose RA (CAS No. 9012-36-6) was purchased as a fine powder from VWR Chemicals LLC. Analytical grade (>99.5%) glycerol (CAS No. 56-81-5) was obtained from Sigma-Aldrich (Darmstadt, Germany). All reagents and materials were used without further purification or modification. Deionized water was degassed in a vacuum oven (SVAC4, Sheldon Manufacturing, Cornelius, Oregon) at 80 °C for 30 min prior to use to minimize dissolved gases that may lead to bubble formation during hydrogel preparation. Two sets of hydrogels were synthesized using 3% and 6% weight/weight (w/w) agarose concentrations. For each agarose group, three glycerol-to-water ratios (w/w) were tested: 25/75, 50/50, and 75/25. Temperature was varied from 10 to 40 ℃ to mimic likely real-world testing, and RH ranges were based on the manufacturer-stated limitations of our environmental chamber (Memmert HPP110eco Climate Chamber 108L, 115V). A low (10%–20%), medium (40%–60%), and high (80%–90%) RH setting was tested for each sample/temperature combination.

For each solution, glycerol was added to the degassed, deionized water and mixed thoroughly, followed by a gradual addition of agarose powder under continuous stirring to prevent any clumping. This solution was chilled to allow hydration of agarose before heating, again reducing the chance of clumping and ensuring uniform dispersion. The homogenous mixture was then heated in a microwave to approximately 80 °C to hydrate and prime the agarose for cooling and gelation, then sonicated at 80 ℃ to remove trapped air and keep the solution liquid during handling. Once clear, the solution was finally poured into 60-mm petri dishes and allowed to cool, entangle, and solidify at room temperature. Once solidified, the initial mass of the sample was recorded and placed in the environmental test chambers set to conditions listed in Table 1. Full sample conditions for all 72 conditions for 216 samples (including results) can be found in Appendix A.

Table 1. Environmental test chamber conditions.

Agarose	25% glycerol	50% glycerol	75% glycerol
3%	10–40 °C + L/M/H RH	10–40 °C + L/M/H RH	10–40 °C + L/M/H RH
6%	10–40 °C + L/M/H RH	10–40 °C + L/M/H RH	10–40 °C + L/M/H RH

Notes: Temperature range: 10, 20, 30, and 40 °C; RH: low (10%–20% RH), medium (40%–60% RH), and high (80%–90% RH).

Samples were weighed again after 24 h of exposure to their respective environmental conditions, or once chamber humidity readings stabilized at the set point indicating equilibrium. Water retention was calculated using Equation 1.

$$\text{Water Loss (\%)} = \frac{M_0 - M_t}{M_0} \times 100\%, \quad (1)$$

where

M_0 = initial mass

M_t = final mass at equilibrium

Finally, for each condition, the mean water loss and standard deviation were calculated across three identical samples to assess the variance.

3. Results and Discussion

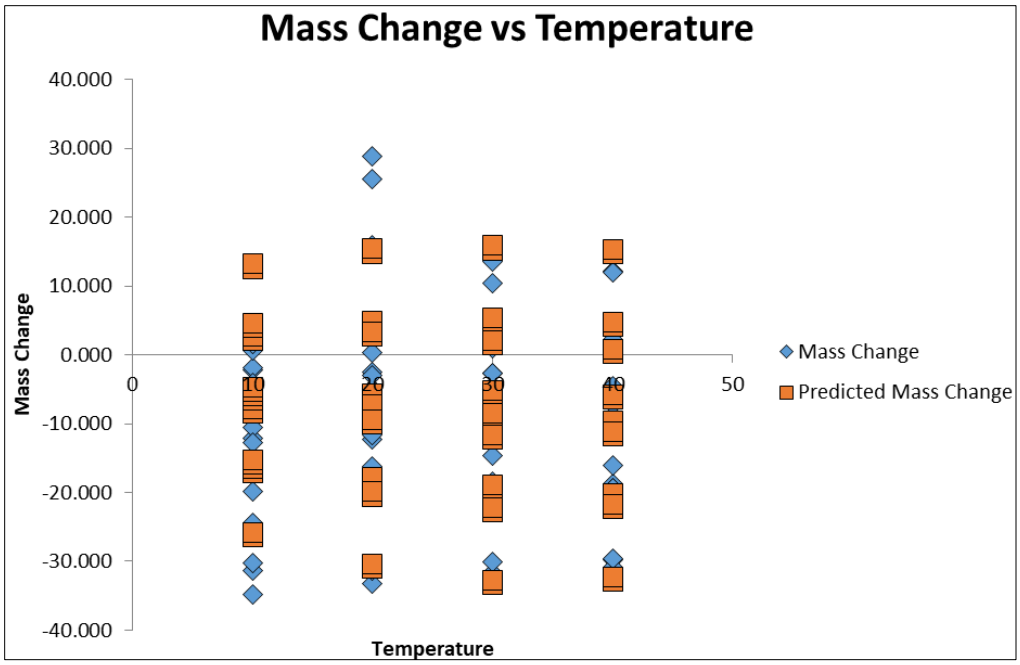
Figure 1 shows mass change versus each variable. As clearly shown, both glycerol concentration and RH have a direct linear impact on desiccation rate and have a point where the trendline crosses the zero-point indicating stability (before beginning to increase mass from absorbing moisture from the air).

We performed an analysis of variance (ANOVA) to determine which factors significantly impact desiccation and then built two multilinear generalized least-squares regression models, one with temperature, glycerol concentration, agarose concentration, and set RH, to visualize and predict the relationship between each factor and water gain or loss. ANOVA results are in Table 2. The significant variables at $p < 0.05$ are glycerol concentration and RH, while temperature and agarose concentration were not significant at $p < 0.05$.

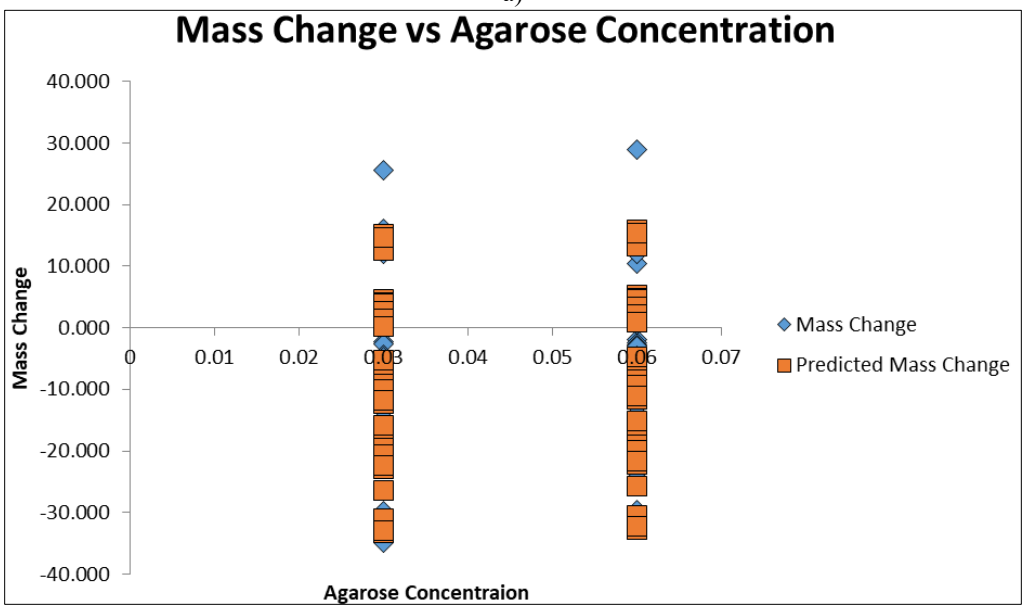
To further elucidate this relationship, Figure 2 shows modeled mass change as a function of only glycerol concentration and RH with an expected nullcline (zero crossing point from Figure 1) where neither desiccation nor absorption should occur. We observe that low humidity or low glycerol tends to cause desiccation, while high humidity and glycerol tend to cause absorption of water from the atmosphere. For most naturally occurring humidity ranges, we have a relative glycerol concentration that should minimize desiccation.

We did not look for edge effects near 0% or 100% RH or glycerol concentrations, as we do not expect to operate in those regions. It is possible that asymptotic behavior might exist, however, we have no reason to believe we would operate outside of the linear region of this model.

We did not look for interactions between temperature and humidity, although it is worth noting that in real-world environments they are likely to move together, e.g., heating a sample will likely change the RH in the immediate vicinity of the heat source due to the temperature change. This humidity change should be considered, and any hydrogel should be formulated to minimize loss at the expected RH at that temperature.

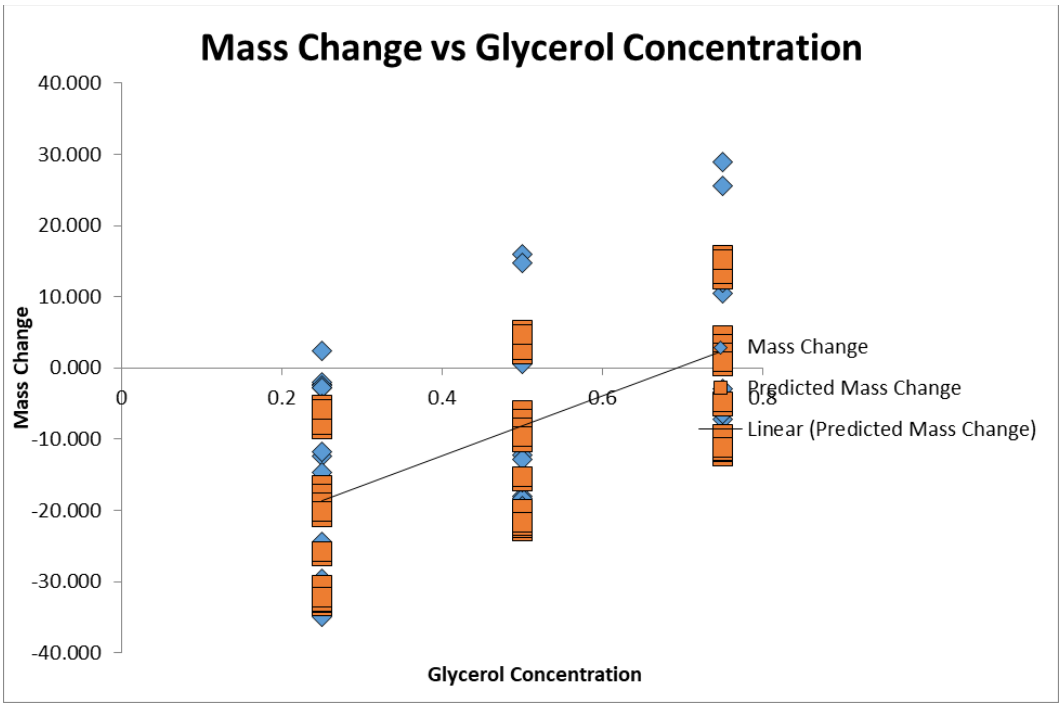


a)



b)

Figure 1. Predicted mass change of hydrogels as a function of a) temperature (° C), b) agarose concentration (% w/w), c) glycerol concentration (% w/w), and d) humidity (% RH): blue, measured; orange, predicted.



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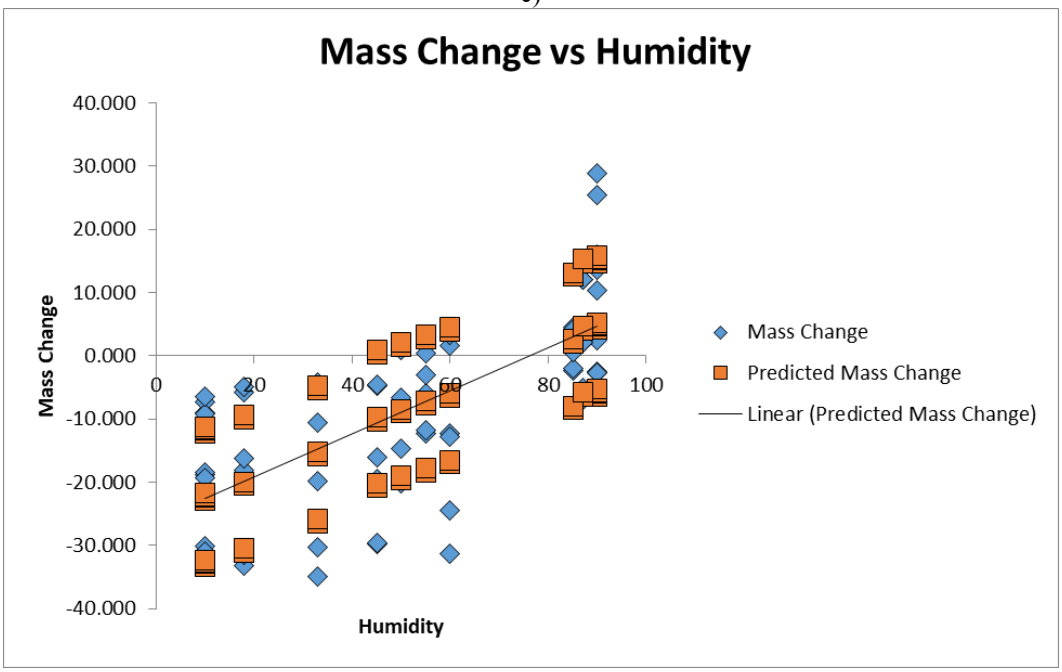

d)

Figure 1. Predicted mass change of hydrogels as a function of a) temperature (° C), b) agarose concentration (% w/w), c) glycerol concentration (% w/w), and d) humidity (% RH): blue, measured; orange, predicted (continued).

Table 2. ANOVA table of the collected desiccation data: humidity and glycerol emerge as significant at $p < 0.05$.

GLS regression results			
Dep. variable:	Mass change	**R-squared:**	0.85
Model:	GLS	**Adj. R-squared:**	0.847
Method:	Least squares	**F-statistic:**	299.2
No. of observations:	216	**Prob (F-statistic):**	9.87E–86
Df residuals:	211		
Df model:	4		

	Coefficient	**Std error**	**t**	**P>|t|**
Intercept	–49.5617	1.944	–25.49	6e–23
Temp	0.0504	0.035	1.451	0.148
Humidity	0.3437	0.013	26.018	2e–23
Glycerol wt%	42.5704	1.88	22.648	3e–20
Agarose	16.8404	25.579	0.658	0.511

Notes: GLS = Generalized Least Squares; Dep. = Dependent; Df = Degrees of Freedom; Adj. = Adjusted; Prob = Probability; Std = Standard

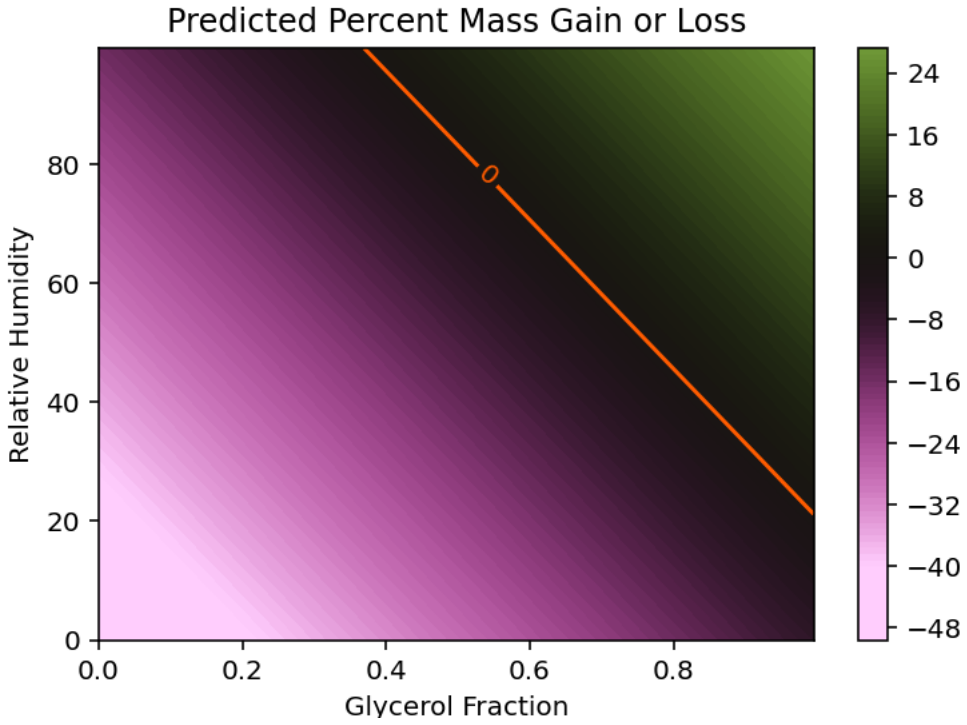

Figure 2. Purple and green indicate predicted mass loss and mass gained, respectively. The orange line shows the expected combinations that should produce a stable phantom.

4. Conclusion and Future Work

The research conducted explored the desiccation behavior of agarose-based hydrogels under varying environmental conditions to determine a design for stable and safe phantom tissue models for EM testing. Our results indicated that glycerol concentration and RH were significant factors that influenced mass change. Agarose concentration and temperature did not significantly influence mass change under our tested ranges. Hydrogels composed of higher glycerol content desiccated less in all humidity conditions, verifying the necessary glycerol concentration to function effectively as a humectant and its role in improving water retention and longevity of surrogate tissue models.

All these findings suggest glycerol-rich hydrogel formulations are best suited for long-lasting surrogates (e.g., phantom head models) for use in controlled environments. Notably, while humidity was carefully controlled for the sake of this study, in most cases it is likely to be a fixed value that the user simply must work within (e.g., ambient humidity in the facility, which may vary by season or location). As a result, Figure 2 can serve to provide as a guide for the ideal water/glycerol fraction that should be used to achieve a stable sample for a given environment.

Future work for this project includes confirming these values for other hydrogel systems, exploring other desiccation prevention methods, and investigating the effects of these high glycerol concentrations on the mechanical and electrical properties of the resultant gels. Further studies will assess how a barrier layer and overall multilayer hydrogel composition will impact mechanical and dielectric properties of hydrogels, ultimately creating a realistic, long-lasting phantom suitable for extensive testing.

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Appendix. Sample Conditions

Table A-1. All 72 sample conditions: changes in mass per samples for all 216 samples with mean and standard deviation per condition.

Temperature (°C)	Relative humidity (%)	Glycerol mass fraction	Agarose mass fraction	Sample 1	Sample 2	Sample 3	Mean mass change	Standard deviation
10	33	0.25	0.03	–34.827	–35.425	–34.379	–34.877	0.524880056
20	18	0.25	0.03	–32.807	–32.438	–34.320	–33.188	0.997091085
30	10	0.25	0.03	–33.200	–32.034	–29.982	–31.739	1.629023075
40	10	0.25	0.03	–35.154	–25.513	–36.320	–32.329	5.931568792
10	60	0.25	0.03	–30.067	–31.782	–31.972	–31.274	1.049199038
20	55	0.25	0.03	–10.792	–11.537	–14.667	–12.332	2.056301179
30	50	0.25	0.03	–15.463	–14.656	–13.948	–14.689	0.757947343
40	45	0.25	0.03	–36.166	–18.943	–34.076	–29.729	9.39877495
10	85	0.25	0.03	–2.513	–2.301	–2.019	–2.278	0.247656065
20	90	0.25	0.03	2.265	2.712	2.258	2.411	0.259956377
30	90	0.25	0.03	–2.496	–2.815	–2.806	–2.706	0.181877211
40	87	0.25	0.03	–7.511	–6.799	–6.673	–6.994	0.452320042
10	33	0.25	0.06	–30.648	–28.986	–31.190	–30.274	1.148619038
20	18	0.25	0.06	–29.434	–31.464	–33.262	–31.387	1.915101641
30	10	0.25	0.06	–30.291	–33.087	–26.966	–30.114	3.064126257
40	10	0.25	0.06	–32.931	–32.524	–27.331	–30.929	3.122565455
10	60	0.25	0.06	–24.665	–24.268	–24.281	–24.405	0.225294072
20	55	0.25	0.06	–14.471	–18.975	–1.692	–11.713	8.965641667
30	50	0.25	0.06	–23.552	–19.508	–17.337	–20.132	3.154104928
40	45	0.25	0.06	–28.949	–28.333	–31.351	–29.545	1.594667489
10	85	0.25	0.06	–2.199	–2.115	–1.566	–1.960	0.343769257
20	90	0.25	0.06	–2.310	–2.778	–2.529	–2.539	0.234001657
30	90	0.25	0.06	–2.434	–2.957	–2.782	–2.724	0.266586281
40	87	0.25	0.06	–4.717	–4.697	–5.705	–5.039	0.576285132
10	33	0.5	0.03	–20.048	–18.954	–20.595	–19.866	0.835604065
20	18	0.5	0.03	–19.514	–18.368	–16.354	–18.079	1.599507839
30	10	0.5	0.03	–22.317	–21.631	–22.006	–21.985	0.343137434
40	10	0.5	0.03	–17.380	–20.007	–18.810	–18.732	1.315530039
10	60	0.5	0.03	–11.139	–12.371	–13.075	–12.195	0.979853739
20	55	0.5	0.03	–5.913	–6.048	–7.362	–6.441	0.80085555
30	50	0.5	0.03	–6.716	–6.777	–6.394	–6.629	0.205871553
40	45	0.5	0.03	–18.943	–20.510	–18.834	–19.429	0.937983838
10	85	0.5	0.03	1.430	1.246	1.418	1.365	0.102734989
20	90	0.5	0.03	15.274	15.612	17.033	15.973	0.933551601
30	90	0.5	0.03	3.084	5.528	4.908	4.507	1.27036978
40	87	0.5	0.03	2.230	1.792	2.322	2.115	0.283094344
10	33	0.5	0.06	–15.963	–0.171	–15.720	–10.618	9.048305264
20	18	0.5	0.06	–17.344	–15.548	–15.669	–16.187	1.003705616
30	10	0.5	0.06	–19.184	–19.248	–16.647	–18.360	1.483522046
40	10	0.5	0.06	–19.736	–18.940	–19.405	–19.360	0.39987183
10	60	0.5	0.06	–11.947	–12.721	–13.823	–12.830	0.942646107
20	55	0.5	0.06	–5.970	–6.360	–5.399	–5.910	0.483562523
30	50	0.5	0.06	–9.024	–8.629	–8.176	–8.610	0.423942351
40	45	0.5	0.06	–17.005	–16.974	–14.070	–16.016	1.685348747
10	85	0.5	0.06	0.625	0.532	0.527	0.561	0.054896122
20	90	0.5	0.06	17.227	12.500	14.436	14.721	2.37658824
30	90	0.5	0.06	4.308	4.592	5.725	4.875	0.74972335
40	87	0.5	0.06	2.223	2.200	2.231	2.218	0.016316276
10	33	0.75	0.03	–4.328	–4.549	–4.332	–4.403	0.126392975
20	18	0.75	0.03	–6.269	–5.585	–5.388	–5.747	0.46272922

Table A-1. All 72 sample conditions: changes in mass per samples for all 216 samples with mean and standard deviation per condition (continued).

Temperature (°C)	Relative humidity (%)	Glycerol mass fraction	Agarose mass fraction	Sample 1	Sample 2	Sample 3	Mean mass change	Standard deviation
30	10	0.75	0.03	–8.288	–9.893	–8.851	–9.011	0.814644639
40	10	0.75	0.03	–7.175	–6.700	–7.892	–7.256	0.599915805
10	60	0.75	0.03	1.649	1.588	1.412	1.550	0.122831603
20	55	0.75	0.03	0.262	0.348	0.411	0.340	0.075101217
30	50	0.75	0.03	1.314	0.900	0.645	0.953	0.337624794
40	45	0.75	0.03	–4.634	–5.033	–4.634	–4.767	0.230217493
10	85	0.75	0.03	5.763	3.972	3.864	4.533	1.066319773
20	90	0.75	0.03	23.873	27.147	25.595	25.539	1.637846008
30	90	0.75	0.03	15.177	12.840	12.844	13.621	1.347701711
40	87	0.75	0.03	11.481	12.559	12.200	12.080	0.54893149
10	33	0.75	0.06	–4.426	–3.248	–4.829	–4.168	0.821473234
20	18	0.75	0.06	–5.377	–4.911	–4.318	–4.868	0.530840001
30	10	0.75	0.06	–9.202	–9.389	–9.026	–9.205	0.181855883
40	10	0.75	0.06	–5.956	–6.937	–6.597	–6.496	0.497871671
10	60	0.75	0.06	3.173	2.767	3.895	3.279	0.571511884
20	55	0.75	0.06	–0.118	–4.866	–3.922	–2.969	2.513283619
30	50	0.75	0.06	1.264	1.322	1.527	1.371	0.138233537
40	45	0.75	0.06	–4.117	–4.961	–4.719	–4.599	0.43462745
10	85	0.75	0.06	5.394	4.428	2.676	4.166	1.377340784
20	90	0.75	0.06	27.372	29.061	30.229	28.887	1.436378211
30	90	0.75	0.06	12.271	10.818	8.176	10.421	2.076203164
40	87	0.75	0.06	11.831	11.744	12.532	12.036	0.432020216

List of Symbols, Abbreviations, and Acronyms

ANOVA	Analysis of Variance
EM	Electromagnetic
H	High
L	Low
M	Medium
RH	Relative Humidity
w/w	Weight/Weight

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