RESEARCH PAPER

TEMPORAL CHANGES IN THE SURFACE TENSION OF HUMAN URINE IN APPARENTLY HEALTHY VOLUNTEERS: A THREE-HOUR OBSERVATIONAL STUDY

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ABSTRACT

Background: Surface tension is an important physicochemical property of biological fluid and is influenced by dissolved solutes, proteins, metabolites, and surfactant - like molecules. Urine is a complex biological fluid containing water, electrolytes, metabolites, organic compounds, and other dissolved substances that determine its physicochemical characteristics. Surface tension represents an important interfacial property influenced by molecular interactions and the presence of surface-active components. The human urine undergoes continuous compositional changes, that may influence surface tension measurements, but short-term temporal variations in its surface tension remain poorly characterized.

Objective: To evaluate the temporal changes in surface tension of first-morning midstream urine samples collected from apparently healthy volunteers over a three-hour observation period.

Methods: A prospective observational study was conducted among apparently healthy hostel residents aged 20–30 years. Seventy-three volunteers (40 females and 33 males) willingly participated in the study. First-morning midstream urine samples were collected in sterile containers as directed to the volunteers. At first specific gravity was evaluated using a refractometer, and then surface tension measurements were performed using a fully automated surface tensiometer based on the Du Noüy ring method. Measurements were recorded at 30, 60, 90, 120, 150, and 180 minutes after collection using the same urine sample. Descriptive statistics were used to summarize the data, like repeated-measures analysis of variance (RM-ANOVA) with Greenhouse-Geisser correction and Bonferroni-adjusted post hoc analysis.

Result: The mean surface tension showed a progressive increase during the observation period. The mean values ranged from 44.68±3.93 mN/m at 30 min to 45.78±4.31 mN/m at 180 min. There was a significant time effect on surface tension (Greenhouse–Geisser corrected $F(2.524,181.696) = 3.216$, $p=0.031$ in the RM-ANOVA. Bonferroni adjusted comparisons showed a significant difference only between 30 and 180 minutes (mean difference −1.108, 95% CI −2.209 to −0.008, $p=0.047$).

Conclusion: Surface tension of human urine undergoes measurable temporal variation during the first three hours after collection. Standardization of the interval between sample collection and analysis is essential when urinary surface tension is evaluated as a laboratory parameter.

Keywords: Urine surface tension, Du Noüy ring method, Fully-automated surface tensiometer; Physicochemical properties, Observational study.

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INTRODUCTION

Urine is one of the most commonly examined biological fluids in the clinical medicine. Conventional urine analysis comprises of physical, chemical and microscopic evaluations to assess the physiological and pathological state of the body, such as evaluation of renal functions, metabolic state and systemic disorders.[1] Apart from the parameters measured in routine urine examination, physicochemical characteristics of urine may provide additional information regarding the molecular composition and fluid behaviour.

Human urine is mainly composed of water along with varying concentration of other constituents, like, inorganic salts, urea, creatinine, organic acids and other metabolites. These constituents determine the physicochemical properties of urine.[2] The urine even after voiding is not chemically static, as exchange of gases, evaporation, alteration of pH, redistribution of molecules, precipitation of solutes etc, processes may occur continuously.[3] These changes may influence the measurement of urinary surface tension after collection.[4]

The Du Nouy ring method is a classical technique to evaluate the surface tension and is basically based on the measurement of force required to detach a platinum-iridium ring form the surface of the liquid.[5] Kyowa automatic surface tensiometer, model DY-300 uses the above principle, which provides standardized measurements of surface tension and interfacial tension of liquids. It is an automatic, convenient and easy to use instrument that also stores the database. The instrument is widely used in physicochemical investigations.

Previous studies have explored urinary surface tension in relation to health and disease[6,7]; however, the data available regarding the short-term temporal variations in the urine surface tension among healthy individuals are very limited. So, to consider the urinary surface tension as a potential laboratory parameter, it is important to establish the baseline temporal behaviour. Therefore, the present study is designed to evaluate the changes in the surface tension of first morning mid-stream urine samples from apparently healthy volunteers over a three-hour observational period using repeated measurements.

Aim:

To evaluate the temporal changes in the surface tension of first morning mid-stream urine during a three-hour observational period.

Materials and Methods:

Study Design and Study Setting:

A prospective observational study was conducted over a period of 9 months at the Vikriti Vigyan Laboratory of Sir Sunderlal Hospital, Banaras Hindu University (BHU), Varanasi.

Study participants:

Apparently healthy hostel residents of BHU aged between 20 and 30 years were selected for the study. Hostel residents were selected to reduce variability associated with dietary and lifestyle factors.

A total of 76 volunteers were approached for participation. The individuals willing to participate were selected. Only three male candidates lacked willingness to participate in the study and were excluded. Therefore, a total of 73 healthy volunteers were included in the final analysis, comprising of 40 female participants and 33 male participants.

Inclusion criteria:

Participants fulfilling the following criteria were included:

A. Apparently healthy individuals aged 20–30 years.
B. Hostel residents.
C. Willingness to participate in the study.
D. Availability of first-morning midstream urine sample.
E. Having no recent history of any drug intake.

Sample size calculation:

Since the objective of the study was to estimate the surface tension, sample size was calculated using the formula for estimation of a single population mean. Assuming a Standard deviation of 6 dyne/cm based on previously published literature[8] and a 95% confidence level, the required sample size was determined using the formula[9]-

$n = (Z\alpha/2^2\ \sigma^2)/d^2$

where:

n = required sample size

$Z\alpha/2$ = standard normal value at 95% confidence level (1.96)

σ = expected standard deviation (6 mN/m) based on previous literature

d = allowable error (1.5 mN/m)

Therefore, estimating the population mean (single) after substitution of values:

$n = (Z\alpha/2^2\ \sigma^2)/d^2$

$n = (1.96)^2\ (6)^2 / (1.5)^2$

n= 61.44

The minimum required sample size was calculated as 62 participants. Therefore, inclusion of 62 volunteers was considered adequate to estimate the mean surface tension with acceptable precision. A total of 73 participants were included in the final analysis, exceeding the calculated requirement.

Urine Sample Collection:[10]

First-morning midstream Urine samples were collected from the participants in sterile containers which was provided to them beforehand, following standard clean-catch collection procedures.

The samples were then bought to the laboratory and maintained at room temperature. Before the commencement of the evaluation, the required amount of urine was transferred in a petri dish (provided by the surface tensiometer). The same urine sample was used for measuring surface tension in all the six-time intervals (30, 60, 90, 120, 150 and 180 mins from its collection) without transferring it into a separate container.

The temperature and humidity were recorded at the beginning of the experiments & measurement were performed under the same laboratory conditions. The surface tension of RO water was measured each day before commencement of the observation to obtain accuracy. The normal surface tension of RO water was 72 mN/m.

Surface Tension Measurement:

Surface tension was measured using an Automatic surface tensiometer (KYOWA, DY-300) based on the Du Nouy ring method. It is a fully automated, user-friendly surface tensiometer.

The Du Novy method determines surface tension by measuring the force required to detach a platinum-iridium ring from the liquid surface and automatically calculates and displays the surface tension corresponding to that force[11]. Measurements were expressed in mN/m. After each reading the platinum ring was sterilised by glow lamp and reused again for successive evaluations.Observation Schedule:

The first measurement was performed 30 minutes after urine collection. Subsequent measurements were obtained from the same sample at:

Observation	Time after collection
T1	30 minutes
T2	60 minutes
T3	90 minutes
T4	120 minutes
T5	150 minutes
T6	180 minutes

A total of six surface tension readings were obtained for each participant.

Statistical Analysis[12]:

Data were analysed using appropriate statistical methods. Descriptive statistics were expressed as mean ± standard deviation. Temporal changes in surface tension were evaluated using repeated-measures analysis of variance (RM-ANOVA). Mauchly's test was used to assess the assumption of sphericity. Since the assumption was violated, the Greenhouse–Geisser correction was applied. Post-hoc comparisons were performed using Bonferroni adjustment. A p-value <0.05 was considered statistically significant.

Statistical analysis was performed by an experienced statistician.

Results:

Participant Characteristics:

A total of 73 apparently healthy volunteers were included.

Characteristic	Number
Total participants	73
Female	40
Male	33
Age group	20–30 years

Observation Table:

Sl. No.	Age	Sex	Date of Exp.	Collection Time (AM)	Specific Gravity (g/cm2)
1	23 years	F	30-10-2025	7:30	0.999
2	22 years	F	30-10-2025	7:45	1.006
3	24 years	F	30-10-2025	8:00	1.009
4	25 years	M	03-11-2025	8:30	1.005
5	26 years	M	03-11-2025	8:45	1.014
6	23 years	F	04-11-2025	9:35	1.014
7	22 years	F	07-11-2025	9:15	1.01
8	24 years	F	07-11-2025	8:55	1.007
9	23 years	F	08-11-2025	9:00	1.011
10	26 years	F	08-11-2025	9:10	1.002
11	21 years	F	11-11-2025	9:05	0.999
12	20 years	F	11-11-2025	9:10	1.004
13	26 years	F	12-11-2025	8:10	1.003
14	26 years	F	12-11-2025	8:10	1.006
15	21 years	F	14-11-2025	8:50	1.015
16	24 years	F	14-11-2025	9:00	0.999
17	26 years	M	18-11-2025	7:40	1.007
18	23 years	M	18-11-2025	7:25	1.006
19	24 years	M	10-02-2026	7:25	1.003
20	22 years	F	11-02-2026	7:47	1.004
21	23 years	M	12-02-2026	7:20	1.016
22	23 years	F	13-02-2026	7:25	1.007
23	23 years	M	14-02-2026	7:30	1.017
24	26 years	F	16-02-2026	7:57	1.013
25	26 years	M	17-02-2026	7:35	1.02
26	28 years	M	18-02-2026	8:16	1.02
27	27 years	F	19-02-2026	7:33	1.003
28	24 years	M	21-02-2026	7:21	1.007
29	24 years	F	24-02-2026	7:25	1.027
30	22 years	F	25-02-2026	7:51	1.012
31	23 years	F	26-02-2026	7:56	1.002

32	29 years	F	28-02-2026	7:46	1.022
33	22 years	M	10-03-2026	7:20	1.026
34	22 years	M	11-03-2026	7:21	1.003
35	22 years	F	12-03-2026	7:17	1.01
36	21 years	M	13-03-2026	7:09	1.003
37	22 years	F	14-03-2026	7:20	1.002
38	27 years	F	17-03-2026	7:20	1.004
39	26 years	M	18-03-2026	7:22	1.027
40	25 years	M	19-03-2026	7:29	1.004
41	22 years	F	25-03-2026	7:15	1.002
42	23 years	M	26-03-2026	7:27	1.015
43	23 years	M	30-03-2026	7:26	1.011
44	23 years	M	01-04-2026	7:25	1.014
45	23 years	F	06-04-2026	7:31	1
46	24 years	M	07-04-2026	7:17	1.003
47	21 years	F	08-04-2026	7:16	1.006
48	23 years	M	09-04-2026	7:10	1.012
49	23 years	M	10-04-2026	7:33	1.018
50	24 years	F	13-04-2026	7:26	1.004
51	21 years	F	15-04-2026	7:10	1.007
52	23 years	M	16-04-2026	7:25	1.002
53	23 years	F	17-04-2026	7:24	1.015
54	25 years	M	20-04-2026	7:20	1.007
55	25 years	F	21-04-2026	8:10	1.006
56	25 years	M	22-04-2026	8:43	1.013
57	24 years	M	23-04-2026	7:28	1.002
58	23 years	M	25-04-2026	7:29	1.007
59	23 years	F	28-04-2026	8:39	1.017
60	25 years	F	12-05-2026	7:05	1.006
61	24 years	M	13-05-2026	7:07	1.013
62	25 years	F	14-05-2026	7:02	1.002
63	21 years	M	15-05-2026	7:59	1.014
64	22 years	F	16-05-2026	7:12	1.017
65	25 years	M	18-05-2026	7:20	1.02
66	25 years	F	19-05-2026	7:03	1.002
67	25 years	M	20-05-2026	7:50	1.014
68	24 years	F	23-05-2026	7:08	1.003
69	24 years	M	25-05-2026	7:32	1.018
70	22 years	F	26-05-2026	7:02	1.002
71	25 years	M	27-05-2026	7:34	1.025
72	22 years	M	29-05-2026	7:10	1.02
73	21 years	F	10-06-2026	7:03	1.009

Table 1: Demographic details and specific gravity of urine samples of study participants

Sl. No.	**Surface Tension of the urine sample at 30 mins interval (mN/m)**					
	30 mins	**60 mins**	**90 mins**	**120 mins**	**150 mins**	**180 mins**
1	46.45	40.38	38.66	45.03	41.67	51.73
2	45.1	44.02	44.78	43.8	42.72	39.38
3	38.73	33.65	35.55	35.97	35.76	33.44
4	48.06	38	4.089	39.07	43.37	48.83
5	49.66	47.25	43.44	43.01	45.17	45.5
6	41.93	43.98	43.01	42.79	42.9	43.33
7	41.08	38.41	40.44	40.76	40.76	40.87
8	48.27	39.06	39.49	37.78	38.63	40.88
9	41.29	43.3	42.18	41.18	40.18	43.67
10	45.99	48.08	49.18	42.08	42	42.52
11	44.48	40.81	41.99	40.59	42.1	44.92
12	48.72	52.48	51.59	47.63	45.55	46.09
13	43.81	44.25	40.04	42.3	41.01	41.76
14	40.67	42.61	41.42	40.56	40.67	40.89
15	41.39	41.39	40.74	39.67	38.6	39.78
16	37.28	50.74	47.87	40.27	43.18	42.21
17	37.04	54.91	55.02	48.71	50.36	51.69
18	40.89	44.99	42.83	43.04	44.67	43.91
19	53.83	55.06	56.18	56.96	57.41	57.75
20	40.26	40.91	41.55	42.74	43.5	44.8
21	49.62	50.5	51.28	51.72	25.02	52.16
22	45.56	46.66	46.66	46.47	46.98	47.09
23	45.78	46.33	46.87	46.98	47.42	47.53
24	45.56	45.24	45.02	45.02	44.88	44.88

25	44.69	44.8	44.69	44.26	44.47	44.26
26	45.56	45.75	46.00	46.11	46.33	46.44
27	44.47	43.93	43.71	44.26	44.04	44.37
28	49.51	51.81	51.83	51.61	51.61	51.61
29	42.09	41.34	41.01	41.01	41.01	40.69
30	48.74	48.96	49.18	49.40	49.51	49.51
31	34.10	36.00	36.42	36.84	36.95	37.16
32	40.48	39.83	39.62	39.40	39.19	39.19
33	46.00	45.89	45.89	45.35	45.35	45.13
34	50.61	50.83	51.06	51.28	51.50	51.61
35	44.47	46.66	46.87	47.75	47.86	47.86
36	48.74	49.79	50.61	50.83	50.95	51.28
37	53.50	51.5	51.83	52.28	53.05	52.94
38	44.04	41.96	41.55	41.88	42.09	42.31
39	43.93	45.02	45.02	44.58	44.8	44.58
40	51.72	53.61	54.72	54.61	54.72	54.95
41	45.78	44.15	44.26	44.8	45.13	45.24
42	47.2	46.98	46.98	46.76	46.76	46.44
43	52.16	51.83	51.72	50.95	50.72	50.61
44	47.75	48.96	49.18	50.61	47.31	46.87
45	42.20	43.93	44.69	44.91	45.35	45.56
46	46.11	47.75	48.3	48.3	48.3	48.74
47	40.15	44.69	45.78	46.11	46.44	46.66
48	42.63	43.93	44.15	44.47	44.58	44.8
49	47.86	47.64	47.53	47.2	46.98	46.76
50	42.63	45.24	46	46.66	46.66	46.76
51	39.13	39.94	39.62	39.73	39.73	39.62
52	44.04	46.22	46.55	47.31	47.64	47.75
53	36.31	37.91	38.23	38.12	38.12	38.12
54	46.55	46.22	46.33	46.44	46.66	47.86
55	43.93	44.8	45.02	44.69	43.82	44.69
56	45.56	46.66	47.09	47.09	47.75	46.76
57	47.86	49.29	49.95	50.95	51.61	51.39
58	49.4	49.51	49.73	49.73	49.84	49.95
59	40.8	41.55	41.88	41.88	42.20	42.52
60	42.63	45.89	46.11	46.11	46.44	47.75
61	42.52	44.69	44.35	45.56	45.89	46.55
62	46.66	47.09	47.75	48.3	49.29	49.73
63	42.42	45.45	45.78	46.44	47.2	47.09
64	47.09	47.97	48.3	48.08	47.97	47.86
65	43.93	44.37	44.69	43.29	44.15	44.04
66	44.90	45.89	46.76	47.09	45.67	47.97
67	46.44	47.86	47.86	47.97	47.75	47.97
68	45.67	45.89	46	46.87	46.22	46.44
69	46.55	47.2	47.31	46.98	47.53	47.31
70	42.2	44.58	45.78	45.67	45.89	46.22
71	45.13	45.02	44.8	45.78	44.37	43.5
72	42.09	43.17	43.61	44.04	44.04	43.93
73	40.91	39.94	39.94	39.73	39.51	39.3

Table 2: Detailed observation of urinary surface tension at six-time interval for each Volunteers.

Repeated Measures Analysis of Variance (RM-ANOVA): A repeated-measures analysis of variance was performed with an objective to determine whether the surface tension of urine changed significantly across the six observation periods.

Descriptive Analysis:

Temporal Changes in Urinary Surface Tension: The mean scores across the six assessment points demonstrated relatively stable values with a slight increasing trend over time. The descriptive statistics are presented below:

Time after collection	Mean ± SD (mN/m)	Standard deviation	N
30 minutes	44.68	± 3.93	73
60 minutes	45.38	± 4.26	73
90 minutes	45.46	± 4.29	73
120 minutes	45.27	± 4.21	73
150 minutes	45.38	± 4.20	73
180 minutes	45.78	± 4.31	73

Table 3: Mean Surface tension values with standard deviation at different observation periods.

highest mean
value was observed at 180 minutes (45.78 ± 4.31 mN/m).

Assessment of Sphericity:

Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated
($W = 0.049$, $\chi^2 = 210.779$, $df = 14$, $p < 0.001$).
Therefore, Greenhouse–Geisser correction was applied for interpretation of within-subject effects. The Greenhouse–Geisser epsilon was **0.505**.

Within-Subject Effects: The repeated measures ANOVA demonstrated a statistically significant effect of time on the outcome measure. Using the Greenhouse–Geisser correction, the within-subject effect was significant:**$F(2.524, 181.696) = 3.216$**
$p = 0.031$

The Huynh–Feldt correction yielded similar findings:
$F(2.623, 188.838) = 3.216$
$p = 0.030$

These results indicate that the mean scores changed significantly across the six assessment periods.

Post-hoc Pairwise Comparisons:

Bonferroni-adjusted pairwise comparisons were performed to identify specific differences between observation periods.

A statistically significant difference was observed only between:

Comparison	Mean Difference	95% CI	p value
30 minutes and 180 minutes	-1.108	-2.209 to -0.008	0.047

No other pairwise comparisons showed statistically significant differences after *Bonferroni correction ($p > 0.05$).*

Figure 1: Mean surface tension values of urine samples measured at six-time intervals after collection. The graph demonstrates a gradual increase in urinary surface tension from 30 minutes to 180 minutes, with the highest mean value observed at the final observation point. Values are expressed as Mean ± SD, n=73

The repeated measures analysis revealed a modest but statistically significant improvement in the outcome variable over the six measurement occasions. Although the overall within-subject effect was significant, post-hoc analysis showed that the meaningful change occurred primarily between the first and sixth assessments. The observed increase of approximately 1.1 units suggests a gradual improvement over time. The violation of the sphericity assumption was appropriately addressed through Greenhouse–Geisser correction, ensuring valid statistical inference.

Discussion:

The present study evaluated the temporal changes in the urinary surface tension of first morning mid-stream urine samples, collected from apparently healthy young adults. The findings demonstrate that urinary surface tension does not remain completely static after collection. Gradually it undergoes measurable changes during a three-hour observation period[4,11].

The urinary surface tension was measured using Du Nouy ring method. In this technique, a ring made of platinum is immersed in the liquid and then gradually pulled through the liquid-air interface.

The maximum force required to detach the ring from the liquid surface is automatically recorded in the fully automated surface tensiometer. The force required to detach the ring from the surface is directly proportional to the surface tension of the liquid. Measurements were performed at room temperature using undiluted urine sample. The Du Nouy method is widely used for biological fluids because it is simple, reproducible as well as sensitive to changes in the interfacial composition due to solutes, proteins, surfactant-like molecules[5]. There was increase in the mean surface tension of urine from 44.68±3.93mN/m at 30 minutes to 45.78±4.31 mN/m at 180 minutes. Although the magnitude of change was small, the repeated measures analysis showed a statistically significant effect of time. The significant difference between the earliest i.e, first observation (at 30 minutes) and final observation (at 180 min) suggests that changes occur gradually rather than immediately after urine collection.

The surface tension at the liquid surface is influenced by the intermolecular forces which depends on the composition of dissolved substances. Urine comprises of various substances such as electrolytes, urea, creatinine, organic acids, proteins and other metabolites that contribute to its physicochemical characteristics[13], changes in the distribution of these components after collection may influence interfacial properties. After voiding, the urine continues to undergo several physicochemical modifications including exchange of dissolved gases with the atmosphere, evaporation of volatile components, alteration of pH, redistribution of surface-active molecules along with possible changes in solute interactions. The mechanism may explain the observed temporal increase by gradually modifying the forces acting at the liquid-air interface[14].

As surface tension of liquid depends on the cohesive forces between the molecules at the air-liquid interface. Pure water has high surface tension whereas urinary surface tension is lower because it contains surface-active solutes that adsorb at the interface[15]. With time, the collected samples can undergo molecular changes like evaporation and concentration effect-in which water evaporates leaving behind salt, urea, creatinine, protein etc in high concentration, reducing surface-active impurities; Degradation or volatilization of surfactant- as fresh urine is comprise of organic surfactant such as urea, proteins, phospholipids, the loss of which causes rise in surface tension due to liquid interface clearance and its resemblance with pure water. CO_2 loss and Ph changes- as fresh urine contain carbon dioxide and bicarbonate ion, which act as natural buffer system. The CO_2 lost in the atmosphere and shifting of hydrogen ion from breakdown of bicarbonate, directly cause the pH of the urine to become more alkaline. Also, the escape of volatile ammonia gas and loss of amphiphilic molecules with time alters the ionic composition and balance of the liquid. As a result, there is change in the ionic strength and reduced adsorption, the strong cohesive forces may take over the charge resulting in increase of surface tension with time.[7,14,16]

The selection between 20-30 years hostel resident of both gender as healthy volunteers provided an advantage by reducing the chances of variations related to age, sex, dietary habits and lifestyle factors[17]. As the participants consumed meals from a common mess facility and also shared a similar living environment, the influences of age, sex and diet on urine composition were minimized.

According to previous studies the biological fluid may show time-dependent changes in surface properties[7,16]. However, most of the earlier studies focused on disease conditions or comparisons between groups than evaluating short-term temporal changes in healthy individuals. Therefore, the present study provides a baseline information regarding the stability of urinary surface tension measurements over a defined period of time.

For the perspective of laboratory use, these findings highlight the importance of controlling the interval between sample collection and urine analysis. If the urinary surface tension is considered for future diagnostic or research applications, standardised timing of measurement will be essential.

Conclusion:

The present study demonstrates that the surface tension of first-morning human urine undergoes significant temporal variation during the first three hours after collection. There was a gradual increase in the urinary surface tension, which was statistically significant mainly between the first and last observation.

These finding indicate that urinary surface tension post collection is not static and influenced by various physicochemical changes. Standardization of sample handling and measurement timing is essential for reliable interpretation of urine surface tension values in future laboratory and research applications.

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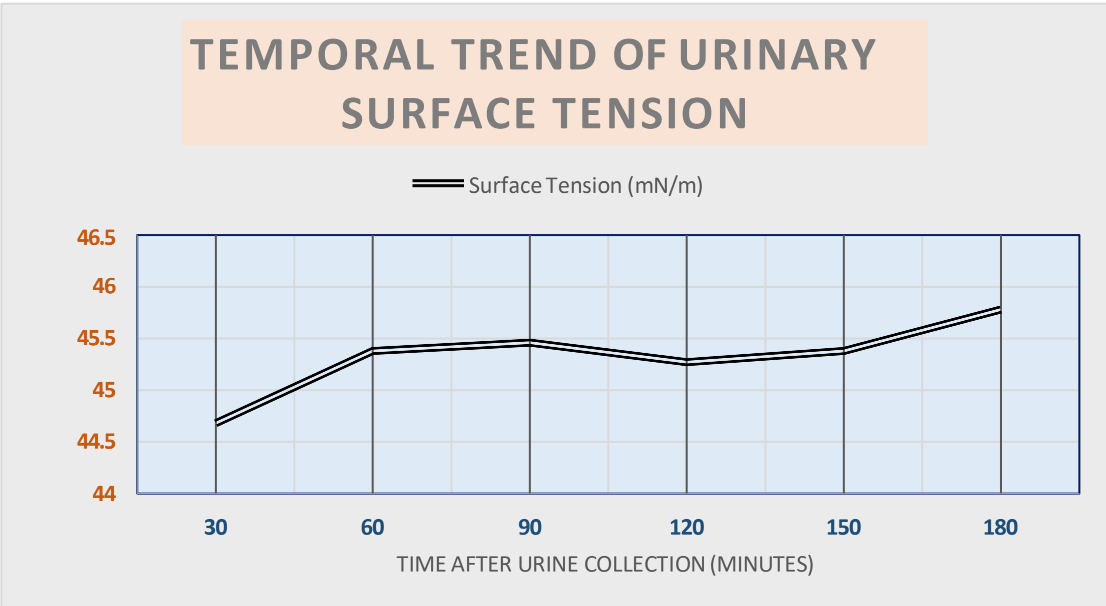

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